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(FILE 'HCAPLUS' ENTERED AT 14:21:05 ON 06 JAN 2003)

L1 1 S (PMS2 OR PMS 2) (W)134

-key terms  
claim 3

L1 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:391731 HCAPLUS

DOCUMENT NUMBER: 136:400586

TITLE: Mammalian cells transfected with defective mismatch repair gene for generating genetically altered antigens and screening of highly immunogenic antigens as vaccines

INVENTOR(S): Nicolaides, Nicholas C.; Grasso, Luigi; Sass, Philip M.

PATENT ASSIGNEE(S): Morphotek Inc., USA

SOURCE: PCT Int. Appl., 76 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO.    | KIND   | DATE     | APPLICATION NO. | DATE     |
|---------------|--|----------|-----------------|----------|
| WO 2002040499 | A1   | 20020523 | WO 2000-US31135 | 20001114 |
| W:            | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |          |                 |          |
| RW:           | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG   |          |                 |          |

AU 2001016030 A5 20020527 AU 2001-16030 20001114

PRIORITY APPLN. INFO.: WO 2000-US31135 A 20001114

AB Dominant neg. alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines animal varieties with novel and useful properties can be prepd. more efficiently than by relying on the natural rate of mutation. These methods are useful for generating genetic diversity within genes encoding for therapeutic antigens to produce altered polypeptides with enhanced antigenic and immunogenic activity. Moreover, these methods are useful for generating effective vaccines. Thus, mouse cell lines transfected with defective human PSM2 gene were prepd. for purpose of the invention.

IT Mutagens

(DNA; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Gene, animal

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(MLH1; defective; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

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IT Gene, animal  
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
(MSH2; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Gene, animal  
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
(PMS1; defective; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Proteins  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(PMS1; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Gene, animal  
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
(PMS2; defective; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Proteins  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(PMS2; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Drug delivery systems  
(carriers; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Suspensions  
(cells; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Proteins  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(gene MLH1; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Proteins  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(gene MSH2; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Antigens  
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)  
(hypermuted; transgenic animals or mammalian cell lines

- comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT Genetic element  
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (leader sequence, 5'-; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT Animal cell  
 (mammalian; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT Gene, animal  
 RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)  
 (mismatch repair; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT DNA repair  
 (mismatch, gene; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT DNA  
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (mutagen; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT Linking agents  
 (poly-; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT Animal  
 Animal cell line  
 Animal virus  
 Bacteria (Eubacteria)  
 Eukaryota  
 Fungi  
 Genetic vectors  
 Human  
 Molecular cloning  
 Mouse  
 Parasitic worm  
 Pathogen  
 Primates  
 Prokaryote  
 Protein sequences  
 Protozoa  
 Rodentia  
 Vaccines  
 cDNA sequences  
 (transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)
- IT Polynucleotides  
 RL: BPN (Biosynthetic preparation); BSU (Biological study,

unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)

(transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Transgene

RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)

(transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Interleukin 2

Reporter gene

mRNA

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT Embryo, animal

(zygote; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT 429062-14-6P, Protein (mouse gene PMS1 isoform 1) 429062-66-8P, Protein MSH2 (mouse gene MSH2 isoform) 429062-89-5P, Protein MLH1 (mouse gene MLH1 isoform) 429063-08-1P, Protein (mouse gene **PMS2-134**)

RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)

(amino acid sequence; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT 429060-80-0, Protein (mouse gene PMS2 isoform 2) 429061-62-1, Protein (mouse gene PMS2 isoform 1)

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(amino acid sequence; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT 429061-04-1 429061-75-6 429062-48-6 429062-76-0 429062-95-3 429063-16-1

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence; transgenic animals or mammalian cell lines comprising defective mismatch repair gene for generating genetically altered antigens and screening for vaccines)

IT 429115-30-0, 1: PN: WO0240499 SEQID: 1 unclaimed DNA 429115-31-1, 2: PN: WO0240499 SEQID: 2 unclaimed DNA 429115-32-2 429115-33-3 429115-34-4

RL: PRP (Properties)

(unclaimed nucleotide sequence; mammalian cells transfected with defective mismatch repair gene for generating genetically altered antigens and screening of highly immunogenic antigens as vaccines)

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REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR  
THIS RECORD. ALL CITATIONS AVAILABLE IN  
THE RE FORMAT

L2 5 S (PMS2 OR PMS 2) (S) 134  
L3 4 S L2 NOT L1

L3 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2003 ACS  
ACCESSION NUMBER: 2002:368239 HCAPLUS  
DOCUMENT NUMBER: 136:364875  
TITLE: Generating hypermutable antibody-producing cells  
using dominant negative alleles of mismatch  
repair genes  
INVENTOR(S): Nicolaides, Nicholas C.; Grasso, Luigi; Sass,  
Philip M.  
PATENT ASSIGNEE(S): Morphotek Inc., USA  
SOURCE: PCT Int. Appl., 75 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO.    | KIND   | DATE     | APPLICATION NO. | DATE     |
|---------------|--|----------|-----------------|----------|
| WO 2002037967 | A1   | 20020516 | WO 2000-US30588 | 20001107 |
| W:            | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |          |                 |          |
| RW:           | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG   |          |                 |          |

AU 2001014708 A5 20020521 AU 2001-14708 20001107

PRIORITY APPLN. INFO.: WO 2000-US30588 A 20001107

AB The invention described herein is directed to the use of random genetic mutation throughout an antibody structure in vivo by blocking the endogenous mismatch repair (MMR) activity of a host cell producing Igs that encode biochem. active antibodies. The invention also relates to methods for repeated in vivo genetic alterations and selection for antibodies with enhanced binding and pharmacokinetic profiles. The invention also provides methods of making hypermutable antibody producing cells by introducing a dominant neg. mismatch repair (MMR) gene such as PMS2 (preferably human PMS2), MLH1, PMS1, MSH2, or MSH2 into cells that are capable of producing antibodies. The dominant neg. allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wildtype **PMS2**). The invention also provides methods in which mismatch repair gene activity is suppressed. This may be accomplished, for example, using antisense mols. directed against the mismatch repair gene or transcripts. These methods are useful for generating genetic

diversity within Ig genes directed against an antigen of interest to produce altered antibodies with enhanced biochem. activity or increased level of antibody prodn. The enhanced rate of mutation can be further augmented using mutagens. The invention demonstrated that a germline truncating mutation in human gene **PMS2** at codon 134 could exert a dominant neg. effect, resulting in biochem. and genetic manifestations of mismatch repair (MMR) deficiency. The invention also demonstrated that dominant neg. mismatch repair gene alleles cause a defect in MMR activity. The invention further demonstrated that MMR and genetic stability can be restored by expressing a MMR gene complementing gene.

- IT Gene, animal
  - RL: BUU (Biological use, unclassified); BIOL (Biological study);
  - USES (Uses)
    - (MLH1; generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Gene, animal
  - RL: BUU (Biological use, unclassified); BIOL (Biological study);
  - USES (Uses)
    - (MSH2; generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Gene, animal
  - RL: BUU (Biological use, unclassified); BIOL (Biological study);
  - USES (Uses)
    - (PMS1; generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Gene, animal
  - RL: BUU (Biological use, unclassified); BIOL (Biological study);
  - USES (Uses)
    - (PMS2; generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Human
  - Primates
  - Rodentia
    - (cell from; generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Eukaryota
  - Mutagenesis
    - (generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Antibodies
  - Immunoglobulins
    - RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
      - (generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Antisense oligonucleotides
  - RL: BUU (Biological use, unclassified); BIOL (Biological study);
  - USES (Uses)
    - (generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Animal cell
  - (mammalian; generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT DNA repair
  - (mismatch; generating hypermutable antibody-producing cells using dominant neg. alleles of mismatch repair genes)
- IT Gene, animal

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RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(mutL; generating hypermutable antibody-producing cells using  
dominant neg. alleles of mismatch repair genes)

IT Mutagens

(mutagenesis using; generating hypermutable antibody-producing  
cells using dominant neg. alleles of mismatch repair genes)

IT Animal

(transgenic; generating hypermutable antibody-producing cells  
using dominant neg. alleles of mismatch repair genes)

IT 424854-96-6, 1: PN: WO0237967 SEQID: 1 unclaimed DNA 424854-97-7,  
2: PN: WO0237967 SEQID: 2 unclaimed DNA 424854-98-8, 3: PN:  
WO0237967 SEQID: 3 unclaimed DNA 424854-99-9, 4: PN: WO0237967  
SEQID: 4 unclaimed DNA 424855-01-6, 6: PN: WO0237967 SEQID: 6  
unclaimed DNA 424855-03-8, 8: PN: WO0237967 SEQID: 8 unclaimed DNA  
424855-05-0 424855-07-2 424855-09-4 424855-11-8

RL: PRP (Properties)

(unclaimed nucleotide sequence; generating hypermutable  
antibody-producing cells using dominant neg. alleles of mismatch  
repair genes)

IT 424855-00-5 424855-02-7 424855-04-9 424855-06-1 424855-08-3  
424855-10-7

RL: PRP (Properties)

(unclaimed protein sequence; generating hypermutable  
antibody-producing cells using dominant neg. alleles of mismatch  
repair genes)

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR  
THIS RECORD. ALL CITATIONS AVAILABLE IN  
THE RE FORMAT

L3 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:851428 HCAPLUS

DOCUMENT NUMBER: 136:1565

TITLE: A method for generating hypermutable cells using  
dominant negative alleles of mismatch repair  
genes

INVENTOR(S): Nicolaides, Nicholas C.; Sass, Philip M.;  
Grasso, Luigi; Vogelstein, Bert; Kinzler,  
Kenneth W.

PATENT ASSIGNEE(S): The Johns Hopkins University, USA; Morphotek  
Inc.

SOURCE: PCT Int. Appl., 59 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE     |
|---|------|----------|-----------------|----------|
| WO 2001088192   | A2   | 20011122 | WO 2001-US15376 | 20010514 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,<br>CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD,<br>GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ,<br>LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,<br>NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,<br>TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ,<br>MD, RU, TJ, TM |      |          |                 |          |

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RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH,  
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE,  
TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD,  
TG

US 2002055106 A1 20020509 US 2001-853646 20010514  
PRIORITY APPLN. INFO.: US 2000-203905P P 20000511  
US 2000-204769P P 20000517

AB Dominant neg. alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepd. more efficiently than by relying on the natural rate of mutation. The enhanced rate of mutation can be further augmented using mutagens. Moreover, the hypermutability of mismatch repair deficient cells can be remedied to stabilize cells or mammals with useful mutations. The invention demonstrated that a germline truncating mutation in human gene **PMS2** at codon 134 could exert a dominant neg. effect, resulting in biochem. and genetic manifestations of mismatch repair (MMR) deficiency. The invention also demonstrated that dominant neg. mismatch repair gene alleles cause a defect in MMR activity. The invention further demonstrated that MMR and genetic stability can be restored by expressing a MMR gene complementing gene.

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(GTBP/MSH6; method for generating hypermutable cells using  
dominant neg. alleles of mismatch repair genes)

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(MLH1; method for generating hypermutable cells using dominant  
neg. alleles of mismatch repair genes)

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(MLH2; method for generating hypermutable cells using dominant  
neg. alleles of mismatch repair genes)

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(MMR (mismatch repair); method for generating hypermutable cells  
using dominant neg. alleles of mismatch repair genes)

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(MSH3; method for generating hypermutable cells using dominant  
neg. alleles of mismatch repair genes)

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(PMS1; method for generating hypermutable cells using dominant  
neg. alleles of mismatch repair genes)

IT Gene, microbial

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(PNP, encoding purine phosphorylase; method for generating  
hypermutable cells using dominant neg. alleles of mismatch repair



genes)

IT Proteins  
mRNA  
RL: ANT (Analyte); ANST (Analytical study)  
(anal. of, encoded by gene of interest; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Remediation  
(bioremediation; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Mutation  
(deletion, in mismatch repair gene MLH1; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Mutation  
(dominant neg., in mismatch repair genes; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Alleles  
(dominant neg., of mismatch repair genes; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Mutagens  
(enhancing mutation in genetic loci with; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Mutagenesis  
(frameshift, in gene PNP; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Antibiotic resistance  
(gene for; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Gene  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(generation of a mutation in; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Reporter gene  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(having a reading frame-shift to monitor hypermutability of cells; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Animal cell  
(hypermutable; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Microsatellite DNA  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(instability, assocd. with defective mismatch repair; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Genetic engineering  
Genetic selection  
Mutagenesis  
Transformation, genetic  
(method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT DNA repair  
(mismatch; method for generating hypermutable cells using

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dominant neg. alleles of mismatch repair genes)

IT Phenotypes  
(of cell or trait, anal. of; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT DNA sequence analysis  
(of gene of interest; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Mutagenesis  
(site-directed, in gene of interest; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Animal  
(transgenic; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT Mutation  
(truncation, in mismatch repair gene PMS2; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT 9030-21-1, Phosphorylase, purine nucleoside  
RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)  
(gene for, having frameshift; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

IT 375400-89-8, 1: PN: WO0188192 SEQID: 1 unclaimed DNA 375400-90-1,  
2: PN: WO0188192 SEQID: 2 unclaimed DNA  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; method for generating hypermutable cells using dominant neg. alleles of mismatch repair genes)

L3 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:636237 HCAPLUS  
DOCUMENT NUMBER: 135:206458  
TITLE: Methods and uses thereof for generating hypermutable yeast for mutagenesis  
INVENTOR(S): Nicolaides, Nicholas C.; Sass, Philip M.; Grasso, Luigi; Vogelstein, Bert; Kinzler, Kenneth W.  
PATENT ASSIGNEE(S): The Johns Hopkins University, USA  
SOURCE: PCT Int. Appl., 59 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO.    | KIND   | DATE     | APPLICATION NO. | DATE     |
|---------------|--|----------|-----------------|----------|
| WO 2001062945 | A1   | 20010830 | WO 2001-US5447  | 20010221 |
| W:            | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |          |                 |          |
| RW:           | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE,  |          |                 |          |

Searcher : Shears 308-4994

09/912697

TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD,  
TG

US 2002123149 A1 20020905 US 2001-788657 20010221  
EP 1259628 A1 20021127 EP 2001-911013 20010221

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC,  
PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRIORITY APPLN. INFO.: US 2000-184336P P 20000223  
WO 2001-US5447 W 20010221

AB The invention claims yeast cells which are hypermutable and methods for producing them. Further, the invention claims yeast strains transformed with dominant neg. alleles and/or truncation alleles of mismatch repair gene mutH homologs, mutS homologs, mutL homologs, mutY homologs, PMS2, plant PMS2, MLH1, MLH3, MSH2, PMSR homologs, PMSL homologs, mammalian PMS2, MSH3, and MSH6. Mutagenesis is mediated by a defective mismatch repair system which can be enhanced using conventional exogenously applied mutagens. Hypermutable yeast cells may be used in biotransformation, bioremediation, and to screen compd. libraries. Yeast cells with the defective mismatch repair system are hypermutable, but after selection of desired mutant yeast strains, they can be rendered genetically stable by restoring the mismatch repair system to proper functionality. Methods for restoring mismatch repair to the hypermutable yeast include transcriptional regulation of the recombinant mutated mismatch repair genes, gene deletion of the same, genetic selection for a functional mismatch repair system, and genetic complementation with wild-type mismatch repair genes. The methods of this invention can be used to obtain desirable mutants in endogenous or exogenous polynucleotides or polypeptides. Detecting desirable mutants can be done by DNA sequence anal., mRNA expression anal., protein analyses, phenotypic analyses, and selection procedures. Desirable mutants include mutations in genes identifying viral antigens, yeast antigens, pharmaceutical targets, and antibiotic resistance genes. An example of the invention is regulated recombinant expression of human PMS134 cDNA and PMSR2 (human PMS2 related gene) in Pichia pastoris. After 48 h of gene PMSR2 expression in a URA+ host, uracil-requiring mutants were detected. Under non-selective growth conditions (in the presence of uracil), the newly-identified ura-mutants were mitotically stable through 5 generations.

IT Alkylating agents, biological  
Oxidizing agents

(DNA; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified);  
BUU (Biological use, unclassified); BIOL (Biological study); PROC  
(Process); USES (Uses)

(MLH1 and MLH3; methods and uses thereof for generating  
hypermutable yeast for mutagenesis)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified);  
BUU (Biological use, unclassified); BIOL (Biological study); PROC  
(Process); USES (Uses)

(MSH2, MSH3, and MSH6; methods and uses thereof for generating  
hypermutable yeast for mutagenesis)

IT Gene, animal

Gene, microbial

Gene, plant

RL: BPR (Biological process); BSU (Biological study, unclassified);

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BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(PMS2; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, microbial  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(PMSR and PMSL homologs; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Transcriptional regulation  
(activation, of recombinant mutated mismatch repair genes; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Complementation (genetic)  
(after loss of recombinant mutated MMR genes; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT DNA sequence analysis  
Phenotypes  
(after mutagenesis; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Proteins, general, biological studies  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(after mutagenesis; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Intercalation  
(agents, DNA; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Remediation  
(bioremediation; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Alleles  
(codon 134 truncation mutation of genes mutS, mutL, and PMS2; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Mutation  
(dominant neg., in mismatch repair genes; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene  
(expression, after mutagenesis; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT mRNA  
RL: ARU (Analytical role, unclassified); ANST (Analytical study)  
(expression, after mutagenesis; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Proteins, specific or class  
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)  
(gene PMS2, N-terminal 133-amino acids of; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Antibiotic resistance  
(genes for, identification; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, animal  
RL: BUU (Biological use, unclassified); BIOL (Biological study);

USES (Uses)  
(hPMSR2, PMSR3, and PMS134; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Recombination, genetic  
(homologous, loss of recombinant mutated MMR genes; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Genetic selection  
(loss of recombinant mutated MMR genes; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Drug screening  
Genetic engineering  
Ionizing radiation  
Mutagenesis  
Mutagens  
Transformation, genetic  
UV radiation  
(methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(mismatch repair (MMR), eukaryotic or prokaryotic; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT DNA repair  
(mismatch, genes for, mutations in; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, microbial  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(mutH homolog; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, plant  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(mutL homolog; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, microbial  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(mutL, homolog; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, microbial  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(mutS, homolog; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Gene, microbial  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(mutY, homolog; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Intercalation

(nucleic acid, agents; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Combinatorial library  
(screening of; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Development, microbial  
(sexual, mating; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Komagataella pastoris  
Yeast  
(transformed; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Mutation  
(truncation, in mismatch repair genes; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT Antigens  
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)  
(viral and yeast, identification of genes for; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT 66-22-8, Uracil, biological studies  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(-requiring mutants, generation of; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT 59-23-4, D-Galactose, biological studies 67-56-1, Methanol, biological studies  
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(inducer of transcription of recombinant mutated MMR genes; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT 62-50-0, EMS  
RL: ADV (Adverse effect, including toxicity); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT 153961-37-6 154211-43-5 158247-45-1 167715-56-2 355482-02-9  
355482-07-4 355482-09-6 355983-13-0, 2: PN: WO0162945 SEQID: 6  
unclaimed DNA 355983-14-1, 3: PN: WO0162945 SEQID: 7 unclaimed DNA  
355983-15-2, 4: PN: WO0162945 SEQID: 8 unclaimed DNA 358018-31-2  
358018-36-7 358018-37-8 358018-38-9  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; methods and uses thereof for generating hypermutable yeast for mutagenesis)

IT 172452-58-3 172452-59-4 358018-28-7 358018-29-8 358018-30-1  
358018-32-3 358018-33-4 358018-34-5 358018-35-6 358018-39-0  
RL: PRP (Properties)  
(unclaimed protein sequence; methods and uses thereof for generating hypermutable yeast for mutagenesis)

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

09/912697

ACCESSION NUMBER: 2000:802345 HCAPLUS  
DOCUMENT NUMBER: 133:359757  
TITLE: Generation of hypermutable organisms using  
dominant negative alleles of the mismatch repair  
gene PMS2  
INVENTOR(S): Nicolaides, Nicholas; Vogelstein, Bert; Kinzler,  
Kenneth W.  
PATENT ASSIGNEE(S): The Johns Hopkins University, USA  
SOURCE: U.S., 21 pp.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

|                        | PATENT NO.   | KIND | DATE     | APPLICATION NO. | DATE     |
|------------------------|--|------|----------|-----------------|----------|
|                        | -----  | ---- | -----    | -----           | -----    |
|                        | US 6146894   | A    | 20001114 | US 1998-59461   | 19980414 |
| PRIORITY APPLN. INFO.: |  |      |          | US 1998-59461   | 19980414 |
| AB                     | Dominant neg. alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. Thus, truncation mutations can be introduced into human wild-type mismatch repair gene <b>PMS2</b> at codons <b>134</b> or <b>424</b> to produce dominant neg. proteins, resulting in hypermutability. The C-terminal region of PMS2 protein is shown to mediate interaction between PMS2 and MLH1 (a mutL homolog involved in the mismatch repair process). By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepd. more efficiently than by relying on the natural rate of mutation. |      |          |                 |          |
| IT                     | Proteins, specific or class<br>RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)<br>(MLH1, interaction with C-terminal region of PMS2; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)  |      |          |                 |          |
| IT                     | Gene, animal<br>RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)<br>(PMS2; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)  |      |          |                 |          |
| IT                     | Proteins, specific or class<br>RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)<br>(gene PMS2 DNA mismatch-repairing; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)  |      |          |                 |          |
| IT                     | Mutagenesis<br>(generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)   |      |          |                 |          |
| IT                     | Mutation<br>(hyper-; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)  |      |          |                 |          |
| IT                     | DNA repair<br>(mismatch; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)  |      |          |                 |          |

Searcher : Shears 308-4994

09/912697

IT 159606-94-7D, Protein (human gene PMS2 reduced), dominant-neg. mutants 304924-00-3, 1-133-Protein (human gene PMS2 reduced) 304924-01-4, 1-423-Protein (human gene PMS2 reduced)  
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
(amino acid sequence; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)  
IT 158247-46-2, GenBank U13696  
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
(nucleotide sequence; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)  
IT 168815-51-8 169110-87-6 307006-91-3, 2: PN: US6146894 PAGE: 10 unclaimed DNA 307006-92-4, 5: PN: US6146894 PAGE: 10 unclaimed DNA  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; generation of hypermutable organisms using dominant neg. alleles of the mismatch repair gene PMS2)

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 14:25:54 ON 06 JAN 2003)

L4 9 S L1 OR L2  
L5 9 DUP REM L4 (0 DUPLICATES REMOVED)

L5 ANSWER 1 OF 9 WPIDS (C) 2003 THOMSON DERWENT  
ACCESSION NUMBER: 2002-500200 [53] WPIDS  
DOC. NO. CPI: C2002-141644  
TITLE: Making a hypermutated antigen, for eliciting an immune response, by introducing into a mammalian cell that expresses a preselected antigen, a polynucleotide comprising a dominant negative allele of a mismatch repair gene.  
DERWENT CLASS: C06 D16  
INVENTOR(S): GRASSO, L; NICOLAIDES, N C; SASS, P M  
PATENT ASSIGNEE(S): (MORP-N) MORPHOTEK INC  
COUNTRY COUNT: 94  
PATENT INFORMATION:

| PATENT NO   | KIND | DATE     | WEEK      | LA | PG |
|---|------|----------|-----------|----|----|
| -----   |      |          |           |    |    |
| WO 2002040499   | A1   | 20020523 | (200253)* | EN | 76 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC |      |          |           |    |    |
| MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW                          |      |          |           |    |    |
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE  |      |          |           |    |    |
| DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG     |      |          |           |    |    |
| KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ     |      |          |           |    |    |
| PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN     |      |          |           |    |    |
| YU ZA ZW  |      |          |           |    |    |
| AU 2001016030   | A    | 20020527 | (200261)  |    |    |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
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Searcher : Shears 308-4994



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|                  |                 |          |
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| WO 2002040499 A1 | WO 2000-US31135 | 20001114 |
| AU 2001016030 A  | WO 2000-US31135 | 20001114 |
|                  | AU 2001-16030   | 20001114 |

FILING DETAILS:

| PATENT NO       | KIND     | PATENT NO    |
|-----------------|----------|--------------|
| AU 2001016030 A | Based on | WO 200240499 |

PRIORITY APPLN. INFO: WO 2000-US31135 20001114

AN 2002-500200 [53] WPIDS

AB WO 200240499 A UPAB: 20020820

NOVELTY - Making (M1) a hypermutated antigen, by introducing into a mammalian cell that expresses a preselected antigen, a polynucleotide (I) comprising a dominant negative allele of a mismatch repair (MMR) gene, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a homogeneous composition (HC) of cultured, hypermutable, mammalian cells comprising a preselected antigen and a dominant negative allele of MMR;

(2) generating a mutation in a gene encoding an antigen of interest, by:

(a) growing (M2) a mammalian cell comprising the gene encoding an antigen of interest and a dominant negative allele of the MMR gene and determining whether the gene encoding an antigen of interest harbors a mutation; or

(b) growing (M3) a cell comprising the gene and (I), and testing the cell to determine whether the cell harbors a mutation in the gene yielding at least one new biochemical feature of the antigen;

(3) a hypermutable transgenic mammalian cell (TC) made by M3;

(4) making (M4) randomly altered forms of a secreted antigen, by introducing a polynucleotide encoding a tagged antigen into a MMR defective cell;

(5) producing (M5) a mutated antigen in a reversibly unstable cell, by introducing into a cell containing a preselected antigen of interest, an inducible expression vector comprising a polynucleotide encoding a dominant negative allele of the MMR gene, inducing the cell to express the dominant negative MMR gene, and detecting at least one new biochemical feature of the antigen;

(6) a polynucleotide molecule (II) for expressing an antigen in a hypermutable cell, comprising an expression cassette comprising a 3' sequence encoding a number of histidine residues, a 5' leader sequence of an expressed gene and a polylinker to allow cloning of a nucleotide sequence encoding a preselected antigen;

(7) producing (M6) a mutated antigen, by introducing a polynucleotide encoding a preselected antigen in the expression cassette of (II), and introducing (II) into a cell comprising a dominant negative allele of MMR gene;

(8) a hypermutated antigen (III) produced by M6; and

(9) an immunogenic composition comprising (III).

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine.

No biological data given.

USE - (III) is useful for eliciting an immune response in an

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animal (claimed). The methods are useful for generating genetic diversity within the gene encoding the therapeutic antigen to produce altered polypeptides with enhanced antigenic and immunogenic activity, and for generating effective vaccines.

ADVANTAGE - The methods are suitable for generating new cell lines and animal varieties with novel and useful properties that can be prepared more efficiently than by relying on the natural rate of mutation. The antigens that are produced are more antigenic, more immunogenic and have beneficial pharmacokinetic properties.  
Dwg.0/4

L5 ANSWER 2 OF 9 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2002-508210 [54] WPIDS

DOC. NO. CPI: C2002-144468

TITLE: Making mammalian cell hypermutable for obtaining a mammalian cell that is resistant to selected microbe by introducing polynucleotide comprising dominant-negative allele of mismatch repair gene into mammalian cell.

DERWENT CLASS: B04 D16

INVENTOR(S): GRASSO, L; NICOLAIDES, N C; SASS, P M

PATENT ASSIGNEE(S): (MORP-N) MORPHOTEK INC

COUNTRY COUNT: 94

PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|-----------|------|------|------|----|----|
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|---------------|----|----------|-----------|----|----|
| WO 2002038750 | A1 | 20020516 | (200254)* | EN | 68 |
|---------------|----|----------|-----------|----|----|

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| RW: | AT | BE | CH | CY | DE | DK | EA | ES | FI | FR | GB | GH | GM | GR | IE | IT | KE | LS | LU | MC |
| MW  | MZ | NL | OA | PT | SD | SE | SL | SZ | TR | TZ | UG | ZW |    |    |    |    |    |    |    |    |

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| W: | AE | AG | AL | AM | AT | AU | AZ | BA | BB | BG | BR | BY | BZ | CA | CH | CN | CR | CU | CZ | DE |
| DK | DM | DZ | EE | ES | FI | GB | GD | GE | GH | GM | HR | HU | ID | IL | IN | IS | JP | KE | KG |    |
| KP | KR | KZ | LC | LK | LR | LS | LT | LU | LV | MA | MD | MG | MK | MN | MW | MX | MZ | NO | NZ |    |
| PL | PT | RO | RU | SD | SE | SG | SI | SK | SL | TJ | TM | TR | TT | TZ | UA | UG | US | UZ | VN |    |
| YU | ZA | ZW |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

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| AU 2001014707 | A | 20020521 | (200260) |  |  |
|---------------|---|----------|----------|--|--|

APPLICATION DETAILS:

| PATENT NO     | KIND | APPLICATION     | DATE     |
|---------------|------|-----------------|----------|
| WO 2002038750 | A1   | WO 2000-US30587 | 20001107 |
| AU 2001014707 | A    | WO 2000-US30587 | 20001107 |
|               |      | AU 2001-14707   | 20001107 |

FILING DETAILS:

| PATENT NO     | KIND       | PATENT NO    |
|---------------|------------|--------------|
| AU 2001014707 | A Based on | WO 200238750 |

PRIORITY APPLN. INFO: WO 2000-US30587 20001107

AN 2002-508210 [54] WPIDS

AB WO 200238750 A UPAB: 20020823

NOVELTY - Making (M1) a mammalian cell hypermutable comprising introducing a polynucleotide with a dominant-negative allele of mismatch repair gene into the mammalian cell which is hypermutable, is new.

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DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a homogenous composition comprising a cultured, hypermutable, mammalian cell with a dominant negative allele of mismatch repair gene.

USE - (M1) is useful for making a mammalian cell hypermutable. The composition is useful for obtaining a mammalian cell that is resistant to a selected microbe. (M1) involves growing a culture of mammalian cells with a dominant negative allele of a mismatch repair gene, exposing the cells to the selected microbe, and selecting the mammalian cell resistant to the selected microbe. The hypermutable cell is resistance to gram-negative and gram-positive microbe, protozoan, bacteria or fungi. The microbial resistance is selected by isolating and testing conditioned medium from the hypermutable cell. The composition is also useful for obtaining a cell comprising a mutation in a gene encoding an antimicrobial activity. (M1) involves growing a culture of mammalian cells with the gene encoding the antimicrobial activity, and a dominant negative allele of a mismatch repair gene, selecting a cell comprising the antimicrobial activity, and determining whether the gene comprises a mutation. The cell is examined to determine whether the gene comprises a mutation by analyzing a nucleotide sequence of the gene or mRNA transcribed from the gene, a protein encoded by gene or its phenotype (all claimed). The composition is useful for identifying antimicrobial agents, microbe-specific toxic molecules, and for producing new phenotypes of the cell. (M1) is useful for creating genetically altered antimicrobial molecules, and also for creating cell lines that manufacture antimicrobial molecules for use in large scale production of antimicrobial agents for clinical studies. (M1) is also useful in cell lines that express known antimicrobial agents to enhance the biochemical activity of the antimicrobial agent.

Dwg.0/6

L5 ANSWER 3 OF 9 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2002-479786 [51] WPIDS

DOC. NO. CPI: C2002-136579

TITLE: Making hypermutable antibody-producing cells for producing antibodies with e.g. enhanced biochemical activity, comprises introducing into a cell a polynucleotide with a dominant negative allele of a mismatch repair gene.

DERWENT CLASS: B04 D16

INVENTOR(S): GRASSO, L; NICOLAIDES, N C; SASS, P M

PATENT ASSIGNEE(S): (MORP-N) MORPHOTEK INC

COUNTRY COUNT: 94

PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|-----------|------|------|------|----|----|
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|---------------|----|----------|-----------|----|----|
| WO 2002037967 | A1 | 20020516 | (200251)* | EN | 75 |
|---------------|----|----------|-----------|----|----|

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| RW: | AT | BE | CH | CY | DE | DK | EA | ES | FI | FR | GB | GH | GM | GR | IE | IT | KE | LS | LU | MC |
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| MW | MZ | NL | OA | PT | SD | SE | SL | SZ | TR | TZ | UG | ZW |
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| W: | AE | AG | AL | AM | AT | AU | AZ | BA | BB | BG | BR | BY | BZ | CA | CH | CN | CR | CU | CZ | DE |
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| DK | DM | DZ | EE | ES | FI | GB | GD | GE | GH | GM | HR | HU | ID | IL | IN | IS | JP | KE | KG |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|

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|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| KP | KR | KZ | LC | LK | LR | LS | LT | LU | LV | MA | MD | MG | MK | MN | MW | MX | MZ | NO | NZ |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|

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|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| PL | PT | RO | RU | SD | SE | SG | SI | SK | SL | TJ | TM | TR | TT | TZ | UA | UG | US | UZ | VN |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|

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| YU | ZA | ZW |
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| AU 2001014708 | A | 20020521 | (200260) |
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APPLICATION DETAILS:

| PATENT NO     | KIND | APPLICATION     | DATE     |
|---------------|------|-----------------|----------|
| WO 2002037967 | A1   | WO 2000-US30588 | 20001107 |
| AU 2001014708 | A    | WO 2000-US30588 | 20001107 |
|               |      | AU 2001-14708   | 20001107 |

FILING DETAILS:

| PATENT NO     | KIND       | PATENT NO    |
|---------------|------------|--------------|
| AU 2001014708 | A Based on | WO 200237967 |

PRIORITY APPLN. INFO: WO 2000-US30588 20001107

AN 2002-479786 [51] WPIDS

AB WO 200237967 A UPAB: 20020812

NOVELTY - Making a hypermutable, antibody-producing cell comprises introducing into a cell, which is capable of producing antibodies, a polynucleotide comprising a dominant negative allele of a mismatch repair gene.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a homogeneous culture of hypermutable, mammalian cells that comprise a dominant negative allele of a mismatch repair gene;

(2) generating a mutation in a gene affecting antibody production in an antibody-producing cell comprising:

(a) growing the cell comprising the gene and a dominant negative allele of a mismatch repair gene, and testing the cell to determine whether the gene harbors a mutation; or

(b) growing the cell comprising the gene and a polynucleotide encoding the dominant negative allele of a mismatch repair gene, and testing the cell to determine whether the cell harbors at least one mutation;

(3) a method where a mammalian cell is made mismatch repair (MMR) defective by introducing a polynucleotide comprising an antisense oligonucleotide targeted against an allele of a mismatch repair gene into a mammalian cell, where the cell becomes hypermutable;

(4) a hypermutable transgenic mammalian cell made by the method of (2b);

(5) reversibly altering the hypermutability of an antibody producing cell by introducing an inducible vector (which comprises a dominant negative allele of a mismatch repair gene operably linked to an inducible promoter) into a cell, and inducing the cell to express the dominant negative mismatch repair gene; and

(6) producing genetically altered antibodies comprising:

(a) transfecting a polynucleotide encoding an immunoglobulin protein into a cell, where the cell comprises a dominant negative mismatch repair gene;

(b) growing the cell to produce a hypermutated polynucleotide encoding a hypermutated immunoglobulin protein;

(c) screening for a desirable property of the hypermutated immunoglobulin protein;

(d) isolating the hypermutated polynucleotide; and

(e) transfecting the hypermutated polynucleotide into a genetically stable cell to produce a hypermutated antibody-producing, genetically stable cell.

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USE - The method is useful for generating genetically altered antibody-producing cell lines with improved antibody characteristics (claimed). In particular, the method is useful for generating genetic diversity within immunoglobulin genes directed against an antigen to produce antibodies with enhanced biochemical activity or for generating antibody-producing cells with increased level of antibody production.

ADVANTAGE - Using the method, antibodies with useful properties can be prepared more efficiently than by relying on the natural rate of mutation.

Dwg.0/6

L5 ANSWER 4 OF 9 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2002-499469 [53] WPIDS

CROSS REFERENCE: 2002-083004 [11]

DOC. NO. CPI: C2002-141391

TITLE: Generating a mutation in a gene using a dominant negative allele of a mismatch repair gene which results in mismatch repair deficiency in cells containing the allele is useful in gene and drug target discovery and recombinant technology.

DERWENT CLASS: B04 D16

INVENTOR(S): GRASSO, L; KINZLER, K W; NICOLAIDES, N C; SASS, P M; VOGELSTEIN, B

PATENT ASSIGNEE(S): (GRAS-I) GRASSO L; (KINZ-I) KINZLER K W; (NICO-I) NICOLAIDES N C; (SASS-I) SASS P M; (VOGE-I) VOGELSTEIN B

COUNTRY COUNT: 1

PATENT INFORMATION:

| PATENT NO     | KIND | DATE     | WEEK      | LA | PG |
|---------------|------|----------|-----------|----|----|
| US 2002055106 | A1   | 20020509 | (200253)* |    | 13 |

APPLICATION DETAILS:

| PATENT NO     | KIND | APPLICATION | DATE                     |
|---------------|------|-------------|--------------------------|
| US 2002055106 | A1   | Provisional | US 2000-203905P 20000512 |
|               |      | Provisional | US 2000-204769P 20000517 |
|               |      |             | US 2001-853646 20010514  |

PRIORITY APPLN. INFO: US 2001-853646 20010514; US 2000-203905P 20000512; US 2000-204769P 20000517

AN 2002-499469 [53] WPIDS

CR 2002-083004 [11]

AB US2002055106 A UPAB: 20020820

NOVELTY - Methods for generating a mutation in a gene of interesting using a dominant negative allele of a mismatch repair gene (D-MMR) under control of an inducible transcriptional regulatory element (ITRE), are new.

DETAILED DESCRIPTION - The method comprises:

(i) generating a mutation in a gene of interest comprising growing a hypermutable mammalian cell comprising the gene and a D-MMR gene under control of an ITRE, and testing the cell to determine whether the gene of interest harbors a mutation;

(ii) generating a mutation in a mammal, comprising growing

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under inducing conditions mammals comprising a D-MMR gene under control of an ITRE and selecting mammals with a new trait acquired during growth, and restoring genetic stability to the mammal by subjecting it to non-inducing conditions;

(iii) generating a mutation in a gene of interest, comprising growing, under inducing condition, mammalian cells comprising a gene of interest and a D-MMR gene under control of an ITRE, contacting the cells with a mutagen, and selecting cells which comprise an altered gene, RNA, polypeptide or phenotypic trait; and

(iv) generating a mutation in a gene of interest, comprising treating cells comprising a gene of interest and a genetic defect in a mismatch repair gene with a mutagen and selecting cells which comprise an altered gene, RNA, polypeptide or phenotypic trait.

INDEPENDENT CLAIMS are also included for the following:

(1) a transgenic mammal made by the above method;

(2) measuring mismatch repair activity of a cell, comprising assaying the function of a gene comprising a polymononucleotide tract in its coding region which disrupts reading frame of the gene downstream of polymononucleotide tract, where function of the gene correlates with reduced mismatch repair activity in the cell; and

(3) a mammal comprising a D-MMR gene under control of an ITRE.

USE - The invention is useful to provide new cell lines that can be used for gene discovery, drug target discovery, recombinant gene mutagenesis or recombinant protein production  
Dwg.0/11

L5 ANSWER 5 OF 9 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2002-083004 [11] WPIDS

CROSS REFERENCE: 2002-499469 [53]

DOC. NO. CPI: C2002-025153

TITLE: Generating mutation in gene using cells which contain defective mismatch repair gene, useful to generate genetically altered mutations with new output traits.

DERWENT CLASS: B04 D16

INVENTOR(S): GRASSO, L; KINZLER, K W; NICOLAIDES, N C; SASS, P M; VOGELSTEIN, B

PATENT ASSIGNEE(S): (GRAS-I) GRASSO L; (KINZ-I) KINZLER K W; (MORP-N) MORPHOTEK INC; (NICO-I) NICOLAIDES N C; (SASS-I) SASS P M; (UYJO) UNIV JOHNS HOPKINS; (VOGE-I) VOGELSTEIN B

COUNTRY COUNT: 96

PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|-----------|------|------|------|----|----|
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|----|------------|----|----------|-----------|-------|
| WO | 2001088192 | A2 | 20011122 | (200211)* | EN 30 |
|----|------------|----|----------|-----------|-------|

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| RW: | AT | BE | CH | CY | DE | DK | EA | ES | FI | FR | GB | GH | GM | GR | IE | IT | KE | LS | LU | MC |
|     | MW | MZ | NL | OA | PT | SD | SE | SL | SZ | TR | TZ | UG | ZW |    |    |    |    |    |    |    |

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|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| W: | AE | AG | AL | AM | AT | AU | AZ | BA | BB | BG | BR | BY | BZ | CA | CH | CN | CO | CR | CU | CZ |
|    | DE | DK | DM | DZ | EC | EE | ES | FI | GB | GD | GE | GH | GM | HR | HU | ID | IL | IN | IS | JP |
|    | KE | KG | KP | KR | KZ | LC | LK | LR | LS | LT | LU | LV | MA | MD | MG | MK | MN | MW | MX | MZ |
|    | NO | NZ | PL | PT | RO | RU | SD | SE | SG | SI | SK | SL | TJ | TM | TR | TT | TZ | UA | UG | US |
|    | UZ | VN | YU | ZA | ZW |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

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|----|------------|---|----------|----------|--|
| AU | 2001061502 | A | 20011126 | (200222) |  |
|----|------------|---|----------|----------|--|

APPLICATION DETAILS:

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| PATENT NO     | KIND | APPLICATION     | DATE     |
|---------------|------|-----------------|----------|
| WO 2001088192 | A2   | WO 2001-US15376 | 20010514 |
| AU 2001061502 | A    | AU 2001-61502   | 20010514 |

FILING DETAILS:

| PATENT NO     | KIND       | PATENT NO    |
|---------------|------------|--------------|
| AU 2001061502 | A Based on | WO 200188192 |

PRIORITY APPLN. INFO: US 2000-204769P 20000517

AN 2002-083004 [11] WPIDS

CR 2002-499469 [53]

AB WO 200188192 A UPAB: 20020823

NOVELTY - Generating a mutation in a gene comprising:

(a) growing a hypermutable mammalian cell containing the gene and a dominant negative allele of a mismatch repair gene under control of an inducible transcriptional regulatory element;

(b) testing the cell to determine if the gene harbors a mutation; and

(c) restoring mismatch repair activity to the cell by decreasing expression of the dominant negative allele, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) generating a mutation in a mammal comprising:

(a) growing mammal(s) containing a dominant negative allele of a mismatch repair gene under control of an inducible transcriptional regulatory element

(b) selecting mammal(s) with a new trait acquired during the growing stage; and

(c) restoring genetic stability to the mammal by subjecting the mammal to non-inducing conditions;

(2) a transgenic mammal made by the above method (1);

(3) generating a mutation in a gene comprising growing mammalian cells containing a dominant negative allele of a mismatch repair gene under control of an inducible regulatory element in inducible conditions, contacting the cells with a mutagen, and selecting cell(s) which comprise an altered gene, mRNA, protein or phenotypic trait;

(4) generating a mutation in a gene comprising treating cells containing the gene and a genetic defect in mismatch repair gene with a mutagen and selecting cell(s) which comprise an altered gene, mRNA, protein or phenotypic trait;

(5) measuring mismatch repair activity of a cell comprising assaying function of a gene containing a polynucleotide tract in its coding region which disrupts the reading frame downstream of the tract, where function of the gene correlates with reduced mismatch repair activity; and

(6) a mammal comprising a dominant negative allele of a mismatch repair gene under control of an inducible transcriptional regulatory element.

USE - The method is used to produce genetically altered organisms to produce new output traits.

Dwg.0/12

L5 ANSWER 6 OF 9 WPIDS (C) 2003 THOMSON DERWENT  
ACCESSION NUMBER: 2001-522820 [57] WPIDS

Searcher : Shears 308-4994

09/912697

DOC. NO. CPI: C2001-156138  
TITLE: Making hypermutable yeast that exhibit novel  
selected output traits for commercial applications,  
comprises introducing polynucleotide containing  
dominant negative allele of mismatch repair gene.  
DERWENT CLASS: B04 D16  
INVENTOR(S): GRASSO, L; KINZLER, K W; NICOLAIDES, N C; SASS, P  
M; VOGELSTEIN, B; ALIS, J M  
PATENT ASSIGNEE(S): (GRAS-I) GRASSO L; (KINZ-I) KINZLER K W; (NICO-I)  
NICOLAIDES N C; (SASS-I) SASS P M; (UYJO) UNIV  
JOHNS HOPKINS; (VOGE-I) VOGELSTEIN B; (ALIS-I) ALIS  
J M  
COUNTRY COUNT: 94  
PATENT INFORMATION:

| PATENT NO   | KIND | DATE     | WEEK      | LA | PG |
|---|------|----------|-----------|----|----|
| WO 2001062945   | A1   | 20010830 | (200157)* | EN | 59 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC<br>MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW   |      |          |           |    |    |
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE<br>DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG<br>KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ<br>PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN<br>YU ZA ZW |      |          |           |    |    |
| AU 2001038558   | A    | 20010903 | (200202)  |    |    |
| US 2002123149   | A1   | 20020905 | (200260)  |    |    |
| US 6454146  | B2   | 20020924 | (200266)  |    |    |

APPLICATION DETAILS:

| PATENT NO     | KIND           | APPLICATION     | DATE     |
|---------------|----------------|-----------------|----------|
| WO 2001062945 | A1             | WO 2001-US5447  | 20010221 |
| AU 2001038558 | A              | AU 2001-38558   | 20010221 |
| US 2002123149 | A1 Provisional | US 2000-184336P | 20000223 |
|               |                | US 2001-788657  | 20010221 |
| US 6454146    | B2 Provisional | US 2000-184336P | 20000223 |
|               |                | US 2001-770348  | 20010126 |

FILING DETAILS:

| PATENT NO     | KIND       | PATENT NO    |
|---------------|------------|--------------|
| AU 2001038558 | A Based on | WO 200162945 |

PRIORITY APPLN. INFO: US 2000-184336P 20000223; US 2001-788657  
20010221; US 2001-770348 20010126

AN 2001-522820 [57] WPIDS  
AB WO 200162945 A UPAB: 20011005  
NOVELTY - Making (M1) a hypermutable yeast (I), comprising  
introducing a polynucleotide (II) containing a dominant negative  
allele (III) of a mismatch repair (MMR) gene, into a yeast, whereby  
the cell becomes hypermutable, is new.  
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for  
the following:  
(1) a homogeneous composition (HC) of cultured, hypermutable,  
yeast comprising (III);

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(2) generating (M2) a mutation in a gene of interest (GI) comprising:

(a) growing a yeast culture containing GI and (III), where the cell is hypermutable, and testing the cell to determine whether GI harbors a mutation; or

(b) growing a yeast cell containing GI and a polynucleotide encoding (III), to create a population of mutated, hypermutable yeast cells, cultivating the population under trait selection conditions, and testing at least one of the cultivated yeast cell to determine whether GI harbors a mutation;

(3) generating (M3) enhanced hypermutable yeast comprising exposing a yeast cell containing (III) to a mutagen, such that an enhanced rate of mutation of the yeast cell is achieved; and

(4) generating (M4) an MMR-proficient yeast with a new output traits comprising growing a MMR-deficient yeast cell containing GI and a polynucleotide encoding (III), to create a population of mutated, hypermutable yeast, cultivating the yeast population under trait selection conditions, testing the yeast cells to determine that GI harbors a mutation, and restoring MMR activity to the yeast cells.

USE - The method is useful to create desirable output traits for commercial applications, using dominant negative alleles of mismatch repair proteins. (I) is useful for production, biocatalysis, bioremediation and drug discovery. (I) is useful in genetic screens for the direct selection of variant subclones that exhibit new output traits. (I) is also useful in manufacturing industry for the generation of new biochemicals, for detoxifying noxious chemicals from by-products of manufacturing processes or those used as catalysts, for remediation of toxins present in the environment including polychlorobenzenes, heavy metals and other environmental hazards for which there is a need to remove them from the environment. (I) is also useful for screening novel mutations in a gene or a set of genes that produce variant siblings that exhibit a new output trait not found in wild type cells. The yeast is also useful for producing increased quantity or quality of protein or non-protein therapeutic molecule e.g., Penicillin G, Erythromycin and Clavulanic acid, by biotransformation. (III) is useful for producing higher quantities of a recombinant polypeptides.

ADVANTAGE - (I) has increased performance characteristics and abilities. The use of (I) in genetic screens bypass the tedious and time-consuming steps of gene identification, isolation and characterization. The yeast strain display novel output features that are suitable for a wide variety of applications.

Dwg.0/0

L5 ANSWER 7 OF 9 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2001-529913 [58] WPIDS

DOC. NO. NON-CPI: N2001-393299

DOC. NO. CPI: C2001-158095

TITLE: Making hypermutable cell, useful for generating hypermutable plants, especially crop plants with new output traits, comprises introducing polynucleotide comprising dominant negative allele of mismatch repair gene into plant cell.

DERWENT CLASS: C06 D16 P13

INVENTOR(S): GRASSO, L; KINZLER, K; NICOLAIDES, N C; SASS, P M; VOGELSTEIN, B

PATENT ASSIGNEE(S): (GRAS-I) GRASSO L; (KINZ-I) KINZLER K; (NICO-I)

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NICOLAIDES N C; (SASS-I) SASS P M; (VOGE-I)  
VOGELSTEIN B  
94

COUNTRY COUNT:

PATENT INFORMATION:

| PATENT NO   | KIND | DATE     | WEEK      | LA | PG |
|---|------|----------|-----------|----|----|
| WO 2001061012   | A1   | 20010823 | (200158)* | EN | 72 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC |      |          |           |    |    |
| MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW                          |      |          |           |    |    |
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE  |      |          |           |    |    |
| DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG     |      |          |           |    |    |
| KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ     |      |          |           |    |    |
| PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN     |      |          |           |    |    |
| YU ZA ZW  |      |          |           |    |    |
| AU 2001026023   | A    | 20010827 | (200176)  |    |    |
| US 2002128460   | A1   | 20020912 | (200262)  |    |    |

APPLICATION DETAILS:

| PATENT NO     | KIND           | APPLICATION     | DATE     |
|---------------|----------------|-----------------|----------|
| WO 2001061012 | A1             | WO 2000-US35397 | 20001228 |
| AU 2001026023 | A              | AU 2001-26023   | 20001228 |
| US 2002128460 | A1 Provisional | US 2000-183333P | 20000218 |
|               |                | US 2000-749601  | 20001228 |

FILING DETAILS:

| PATENT NO     | KIND       | PATENT NO    |
|---------------|------------|--------------|
| AU 2001026023 | A Based on | WO 200161012 |

PRIORITY APPLN. INFO: US 2000-183333P 20000218; US 2000-749601  
20001228

AN 2001-529913 [58] WPIDS

AB WO 200161012 A UPAB: 20011010

NOVELTY - Making a hypermutable cell comprises introducing into a plant cell a polynucleotide comprising a dominant negative allele of a mismatch repair gene, where the cell becomes hypermutable

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a homogeneous composition of cultured, hypermutable, plant cells that comprise a dominant negative allele of a mismatch repair gene;

(2) a hypermutable transgenic plant where at least 50% of the cells of the plant comprise a dominant negative allele of a mismatch repair gene;

(3) generating a mutation in a gene of interest in a plant cell comprising:

(a) growing a hypermutable plant cell comprising the gene of interest and a dominant negative allele of a mismatch repair gene; and

(b) testing the cell to determine whether the gene of interest harbors a mutation;

(4) generating a mutation in a gene of interest in a plant comprising:

(a) growing a plant comprising the gene of interest and a

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polynucleotide encoding a dominant allele of a mismatch repair gene;  
and

(b) testing the plant to determine whether the gene of interest harbors a mutation;

(5) a hypermutable transgenic plant made by the method;

(6) generating a hypermutable plant comprising inhibiting endogenous mismatch repair (MMR) activity of a plant, where the plant becomes hypermutable;

(7) a vector for introducing a dominant negative MMR allele into a plant comprising a dominant negative MMR allele under the transcriptional control of a promoter that is functional in a plant;

(8) isolated and purified polynucleotides encoding:

Arabidopsis PMS2, which comprises a sequence having 147 amino acids fully defined in the specification; or

Arabidopsis PMS134, which has a sequence that is defined in the specification;

(9) isolated and purified proteins comprising Arabidopsis PMS2 or Arabidopsis PMS134; and

(10) determining the presence of a MMR defect in a plant or plant cell comprising:

(a) comparing at least two microsatellite markers in test cells or a test plant to the two (or more) microsatellite markers in cells of a normal plant; and

(b) identifying the test cells or test plant as having a mismatch repair defect if at least two microsatellite markers are found to be rearranged relative to the cells of the normal plant.

USE - The method is useful for generating hypermutable plants. The method is particularly useful for generating or producing new cell lines and varieties. This is particularly useful for agriculturally important crops. The method is also useful for generating crop plants with new output traits and plant cells exhibiting new biochemicals for commercial use.

Dwg.0/13

L5 ANSWER 8 OF 9 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2001-514664 [56] WPIDS

DOC. NO. CPI: C2001-153855

TITLE: Making hypermutable bacteria for biocatalysis, bioremediation and drug discovery, involves introducing polynucleotide comprising dominant negative allele of mismatch repair gene under regulatory sequence control.

DERWENT CLASS: B04 D16

INVENTOR(S): GRASSO, L; KINZLER, K W; NICOLAIDES, N C; SASS, P M; VOGELSTEIN, B

PATENT ASSIGNEE(S): (UYJO) UNIV JOHNS HOPKINS; (GRAS-I) GRASSO L; (KINZ-I) KINZLER K W; (NICO-I) NICOLAIDES N C; (SASS-I) SASS P M; (VOGE-I) VOGELSTEIN B

COUNTRY COUNT: 94

PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
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|-----------|------|------|------|----|----|

|               |    |          |           |    |    |
|---------------|----|----------|-----------|----|----|
| WO 2001059092 | A2 | 20010816 | (200156)* | EN | 68 |
|---------------|----|----------|-----------|----|----|

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| RW: | AT | BE | CH | CY | DE | DK | EA | ES | FI | FR | GB | GH | GM | GR | IE | IT | KE | LS | LU | MC |
|     | MW | MZ | NL | OA | PT | SD | SE | SL | SZ | TR | TZ | UG | ZW |    |    |    |    |    |    |    |

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| W: | AE | AG | AL | AM | AT | AU | AZ | BA | BB | BG | BR | BY | BZ | CA | CH | CN | CR | CU | CZ | DE |
|    | DK | DM | DZ | EE | ES | FI | GB | GD | GE | GH | GM | HR | HU | ID | IL | IN | IS | JP | KE | KG |

Searcher : Shears 308-4994

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KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ  
PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN  
YU ZA ZW  
AU 2001034992 A 20010820 (200175)  
US 2002068284 A1 20020606 (200241)

APPLICATION DETAILS:

| PATENT NO     | KIND           | APPLICATION     | DATE     |
|---------------|----------------|-----------------|----------|
| WO 2001059092 | A2             | WO 2001-US4339  | 20010212 |
| AU 2001034992 | A              | AU 2001-34992   | 20010212 |
| US 2002068284 | A1 Provisional | US 2000-181929P | 20000211 |
|               |                | US 2001-780675  | 20010212 |

FILING DETAILS:

| PATENT NO     | KIND       | PATENT NO    |
|---------------|------------|--------------|
| AU 2001034992 | A Based on | WO 200159092 |

PRIORITY APPLN. INFO: US 2000-181929P 20000211; US 2001-780675  
20010212

AN 2001-514664 [56] WPIDS

AB WO 200159092 A UPAB: 20011001

NOVELTY - Making (M1) a hypermutable bacteria (I), comprising introducing a polynucleotide (II) having a dominant negative allele (III) of a mismatch repair (MMR) gene under the control of an inducible transcription regulatory sequence, into a bacterium, is new. The cell becomes inducibly hypermutable.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a homogeneous composition (HC) of cultured, hypermutable, bacteria comprising (III);

(2) generating (M2) a mutation in a gene of interest (GI), by:

(a) growing bacterial culture comprising GI and (III), where the cell is hypermutable, and testing the cell to determine if GI harbors a mutation; or

(b) growing a bacterium comprising GI and (III), to form a population of mutated bacteria, cultivating the population under trait selection conditions, and testing at least one of the cultivated bacteria to determine whether GI harbors a mutation;

(3) enhancing (M3) the mutation rate of a bacterium, by exposing a bacterium comprising (III) to a mutagen, the mutation rate of the bacterium is enhanced in excess of the rate in the absence of mutagen or (III); and

(4) generating (M4) an MMR-proficient bacterium with a new output trait, by growing a MMR-deficient bacterium comprising a defective MMR gene allele and GI, to form a population of mutated bacteria, cultivating the bacterial population under trait selection conditions, testing at least one of the cultivated bacteria to determine that GI harbors a mutation, and restoring MMR activity to at least one cultivated bacteria.

USE - The method is useful to create desirable output traits for commercial applications, using dominant negative alleles of mismatch repair proteins. (I) is useful for production, biocatalysis, bioremediation and drug discovery. (I) is also useful in manufacturing industry for the generation of new biochemicals

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useful for detoxifying noxious chemicals from by-products of manufacturing processes or those used as catalysts, for remediation of toxins present in the environment including polychlorobenzenes, heavy metals and other environmental hazards for which there is a need to remove them from the environment. (I) is also useful for screening novel mutations in a gene or a set of genes that produce variant siblings that exhibit a new output trait not found in wild type cells. The bacteria are also useful for producing increased quantity or quality of protein or non-protein therapeutic molecule e.g. Penicillin G, Erythromycin and Clavulanic acid, by biotransformation. (III) is useful for producing higher quantities of a recombinant polypeptides.  
Dwg.0/6

L5 ANSWER 9 OF 9 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2000-137587 [13] WPIDS

DOC. NO. NON-CPI: N2000-102905

DOC. NO. CPI: C2000-042344

TITLE: Generating hypermutable cells for research in hereditary nonpolyposis colorectal cancer syndrome comprises introduction of polynucleotide having a dominant negative allele of a mismatch repair gene.

DERWENT CLASS: B04 D16 P14 S03

INVENTOR(S): KINZLER, K W; NICOLAIDES, N; VOGELSTEIN, B

PATENT ASSIGNEE(S): (UYJO) UNIV JOHNS HOPKINS

COUNTRY COUNT: 2

PATENT INFORMATION:

| PATENT NO  | KIND | DATE     | WEEK      | LA | PG |
|------------|------|----------|-----------|----|----|
| CA 2240609 | A1   | 19991014 | (200013)* | EN | 50 |
| US 6146894 | A    | 20001114 | (200060)  |    |    |

APPLICATION DETAILS:

| PATENT NO  | KIND | APPLICATION     | DATE     |
|------------|------|-----------------|----------|
| CA 2240609 | A1   | CA 1998-2240609 | 19980811 |
| US 6146894 | A    | US 1998-59461   | 19980414 |

PRIORITY APPLN. INFO: US 1998-59461 19980414

AN 2000-137587 [13] WPIDS

AB CA 2240609 A UPAB: 20000313

NOVELTY - A method for making a hypermutable cell, comprising introducing into a mammalian cell a polynucleotide having a dominant negative allele of a mismatch repair gene, where the cell becomes hypermutable, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a homogenous composition of cultured, hypermutable, mammalian cells which comprise a dominant negative allele of a mismatch repair gene;

(2) a hypermutable transgenic mammal where at least 50% of the cells of the mammal comprise a dominant negative allele of a mismatch repair gene;

(3) a method for generating a mutation in a gene of interest comprising:

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(a) growing a mammalian cell comprising the gene of interest and a dominant negative allele of a mismatch repair gene, where the cell is hypermutable; and

(b) testing the cell to determine whether the gene of interest harbors a mutation; and

(4) a method of generating a mutation in a gene of interest comprising:

(a) growing a mammal comprising the gene of interest and a polynucleotide encoding a dominant negative allele of a mismatch repair gene; and

(b) testing the mammal to determine whether the gene of interest harbors a mutation.

USE - The method is useful for introducing genes into cells and transgenic animals allowing new cell lines and animal varieties with novel and useful properties to be prepared more efficiently than by relying on the natural rate of mutation. The methods are also useful for research in hereditary nonpolyposis colorectal cancer syndrome in patients.

ADVANTAGE - Once a transfected cell or a colony of transgenic animals have been produced, it can be used to generate new mutations in one or more genes of interest. The methods allow mutations to cells or animals to occur without the need for mutagenic chemicals or radiation which may have secondary harmful effects.

Dwg.0/6

(FILE 'REGISTRY' ENTERED AT 14:28:23 ON 06 JAN 2003)

L8 8 S (QUINILONE OR AMINOGLYCOSIDE OR MAGAININ OR DEFENSIN OR  
E AMINO GLYCOSIDE/CN 5  
E AMINOGLYCOSIDE/CN 5  
E AMINOGLYCOSIDES/CN 5  
E QUINILON/CN  
E ".BETA.-LACTAM"/CN 5  
E ".BETA.-LACTAMS"/CN 5  
E BETA LACTAM/CN 5

FILE 'HCAPLUS' ENTERED AT 14:31:49 ON 06 JAN 2003

L8 8 SEA FILE=REGISTRY ABB=ON PLU=ON (QUINILONE OR AMINOGLYC  
OSIDE OR MAGAININ OR DEFENSIN OR TETRACYCLINE OR  
".BETA.-LACTAM" OR MACROLIDE OR LINCOSAMIDE OR SULFONAMID  
E OR SULPHONAMIDE OR CHLORAMPHENICOL OR NITROFURANTOIN  
OR ISONIAZID)/CN  
L9 1160 SEA FILE=HCAPLUS ABB=ON PLU=ON (MMR OR MISMATCH?  
REPAIR?) (5A) GENE  
L10 13 SEA FILE=HCAPLUS ABB=ON PLU=ON L9 AND (L8 OR ANTIBIOTIC  
OR QUINILONE OR AMINOGLYCOSIDE OR AMINO GLYCOSIDE OR  
MAGAININ OR DEFENSIN OR TETRACYCLIN? OR TETRA CYCLIN? OR  
BETA LACTAM OR MACROLIDE OR LINCOSAMIDE OR SULFONAMIDE  
OR SULPHONAMIDE OR CHLORAMPHENICOL OR NITROFURANTOIN OR  
NITRO FURANTOIN? OR ISONIAZID?)

L11 11 L10 NOT (L1 OR L3)

L11 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:461222 HCAPLUS

DOCUMENT NUMBER: 137:42547

TITLE: Mismatch repair detection applicable for

Searcher : Shears 308-4994

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high-throughput genotyping and mutation  
detection

INVENTOR(S): Cox, David R.; Faham, Malek; Baharloo, Siamak  
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford  
Junior University, USA; The Regents of the  
University of California  
SOURCE: U.S., 18 pp., Cont.-in-part of U.S. Ser. No.  
713,751, abandoned.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 4  
PATENT INFORMATION:

| PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE        |
|---|------|----------|-----------------|-------------|
| US 6406847  | B1   | 20020618 | US 1999-271055  | 19990317    |
| WO 2000055369   | A1   | 20000921 | WO 2000-US6731  | 20000314    |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR,<br>CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,<br>HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,<br>LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO,<br>RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US,<br>UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM<br>RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,<br>DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF,<br>BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG  |      |          |                 |             |
| EP 1175510  | A1   | 20020130 | EP 2000-916338  | 20000314    |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC,<br>PT, IE, SI, LT, LV, FI, RO  |      |          |                 |             |
| JP 2002538840   | T2   | 20021119 | JP 2000-605785  | 20000314    |
| US 2002172966   | A1   | 20021121 | US 2002-72047   | 20020208    |
| US 2003003472   | A1   | 20030102 | US 2002-81771   | 20020220    |
| PRIORITY APPLN. INFO.:  |      |          |                 |             |
|   |      |          | US 1995-4664P   | P 19951002  |
|   |      |          | US 1996-713751  | B2 19960913 |
|   |      |          | US 1999-271055  | A 19990317  |
|   |      |          | WO 2000-US6731  | W 20000314  |
| AB Mismatch Repair Detection (MRD), a novel method for DNA-variation<br>detection, utilizes bacteria to detect mismatches by a change in<br>expression of a marker gene. DNA fragments to be screened for<br>variation are cloned into two MRD plasmids, and bacteria are<br>transformed with heteroduplexes of these constructs. Resulting<br>colonies express the marker gene in the absence of a mismatch, and<br>lack expression in the presence of a mismatch. MRD is capable of<br>detecting a single mismatch within 10 kb of DNA. In addn., MRD can<br>analyze many fragments simultaneously, offering a powerful method<br>for high-throughput genotyping and mutation detection. The method<br>was demonstrated using two puC-derived plasmids, pMF200 and pMF100,<br>which are identical except that there is a 5-bp insertion in the<br>lacZ.alpha. gene of pMF100. The method of mutation detection<br>comprises cloning one copy of the DNA in question in pMF200, the<br>other copy into pMF100. The pMF200 plasmid is cloned in dam- E.<br>coli (no methylation of the plasmid); the pMF100 plasmid is cloned<br>in dam+ E. coli (methylation of plasmid). The plasmids are<br>isolated, linearized, denatured, and reannealed, then digested with<br>MboI and DpnI. E. coli are transformed with the resulting<br>hemimethylated heteroduplexes. The transformants are cultured and<br>.beta.-galactosidase activity detected as usual. If no mutation was |      |          |                 |             |

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present (i.e., no mismatch), no repair occurs and the colonies are blue. If a mutation was present, repair occurs and the lacZ.alpha. mutant is corepaired resulting in colonies with white color. In addn. of LacZ.alpha. gene, the gene for Cre recombinase (Cre) can be also used as the marker gene for bacteria carrying two **antibiotic** selection markers (tetR and strepS genes) flanked by two lox sites. The resulting colonies are **tetracycline** sensitive and streptomycin resistant in the absence of a mismatch and **tetracycline** resistant and streptomycin sensitive in the presence of a mismatch.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 2 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:368640 HCAPLUS

DOCUMENT NUMBER: 136:381348

TITLE: Generating hypermutable mammalian cells using dominant negative alleles of **mismatch repair genes** for isolating antimicrobial agents

INVENTOR(S): Grasso, Luigi; Nicolaides, Nicholas C.; Sass, Philip M.

PATENT ASSIGNEE(S): Morphotek Inc., USA

SOURCE: PCT Int. Appl., 68 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO.    | KIND   | DATE     | APPLICATION NO. | DATE     |
|---------------|--|----------|-----------------|----------|
| WO 2002038750 | A1   | 20020516 | WO 2000-US30587 | 20001107 |
| W:            | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |          |                 |          |
| RW:           | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG   |          |                 |          |

AU 2001014707 A5 20020521 AU 2001-14707 20001107

PRIORITY APPLN. INFO.: WO 2000-US30587 A 20001107

AB The present invention described herein is directed to the use of random genetic mutation of a cell to produce novel **antibiotics** by blocking the endogenous mismatch repair activity of a host cell by introducing a dominant neg. **mismatch repair (MMR) gene** such as PMS2 (preferably human PMS2), MLHI, PMS1, MSH2, or MSH2. The cell can be a mammalian cell that produces an antimicrobial agent naturally, or a cell that is placed under selective pressure to obtain a novel antimicrobial mol. that attacks a specific microbe. Moreover, the invention describes methods for obtaining enhanced antimicrobial activity of a cell line that produces an



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antimicrobial activity due to recombinant expression or as part of the innate capacity of the cell to harbor such activity. An embodiment of the invention described herein is directed to the creation of genetically altered host cells with novel and/or increased antimicrobial prodn. that are generated by a method that interferes with the highly ubiquitous and phylogenetically conserved process of mismatch repair. An example of a dominant neg. allele of a **mismatch repair gene** is the human **gene hPMS2-134**, which carries a truncation mutation at codon 134. The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide contg. the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations that accumulate in cells after DNA replication. Syrian Hamster TK fibroblasts transfected with a mammalian expression vector contg. a novel antimicrobial polypeptide called mlgl and grown in the presence of *Bacillus subtilis* were able to suppress the growth of the microbes. *Escherichia coli* bacterial growth was significantly suppressed in TK-tsl3 cells constitutively expressing the dominant-neg. **mismatch repair gene**, TK-hPMS2-134.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 3 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:256458 HCAPLUS

DOCUMENT NUMBER: 136:289898

TITLE: Targeted gene correction by single-stranded oligonucleotides and its use in gene therapy

INVENTOR(S): Yoon, Kyonggeun; Igoucheva, Olga

PATENT ASSIGNEE(S): Thomas Jefferson University, USA

SOURCE: PCT Int. Appl., 73 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO.   | KIND | DATE     | APPLICATION NO. | DATE       |
|--|------|----------|-----------------|------------|
| WO 2002026967  | A2   | 20020404 | WO 2001-US29909 | 20010925   |
| W: CA, JP, US  |      |          |                 |            |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR |      |          |                 |            |
| US 2002119570  | A1   | 20020829 | US 2001-962628  | 20010925   |
| PRIORITY APPLN. INFO.:   |      |          | US 2000-235226P | P 20000925 |

AB The present invention relates to using single-stranded oligonucleotides that are designed to specifically change a base in a target nucleic acid sequence. This alteration is maintained, expressed and regulated as the normal endogenous gene. Specifically, the present invention uses short deoxyoligonucleotides that are designed to effect a sequence-specific change in a target sequence, thereby generating a predefined alteration in the target sequence. This sequence-specific change is maintained in progeny cells. The present invention therefore solves a long sought need to develop a simple system to effect a genetic change, and to maintaining this genetic change throughout the lifespan of the target cell.

Searcher : Shears 308-4994

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L11 ANSWER 4 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:634531 HCAPLUS

DOCUMENT NUMBER: 136:258038

TITLE: Analysis of the chromosome sequence of the  
legume symbiont *Sinorhizobium meliloti* strain  
1021

AUTHOR(S): Capela, Delphine; Barloy-Hubler, Frederique;  
Gouzy, Jerome; Bothe, Gordana; Ampe, Frederic;  
Batut, Jacques; Boistard, Pierre; Becker, Anke;  
Boutry, Marc; Cadieu, Edouard; Dreano, Stephane;  
Gloux, Stephanie; Godrie, Therese; Goffeau,  
Andre; Kahn, Daniel; Kiss, Erno; Lelaure,  
Valerie; Masuy, David; Pohl, Thomas; Portetelle,  
Daniel; Puhler, Alfred; Purnelle, Benedicte;  
Ramsperger, Ulf; Renard, Clotilde; Thebault,  
Patricia; Vandenbol, Micheline; Weidner, Stefan;  
Galibert, Francis

CORPORATE SOURCE: Laboratoire de Biologie Moleculaire des  
Relations Plantes-Microorganismes, Unite Mixte  
de Recherche (UMR) 215 Centre National de la  
Recherche Scientifique (CNRS), Institut National  
de la Recherche Agronomique, Chemin, Tolosan,  
F-31326, Fr.

SOURCE: Proceedings of the National Academy of Sciences  
of the United States of America (2001), 98(17),  
9877-9882

CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB *Sinorhizobium meliloti* is an  $\alpha$ -proteobacterium that forms  
agronomically important N<sub>2</sub>-fixing root nodules in legumes. We  
report here the complete sequence of the largest constituent of its  
genome, a 62.7% GC-rich 3654,135-bp circular chromosome. Annotation  
allowed assignment of a function to 59% of the 3341 predicted  
protein-coding ORFs, the rest exhibiting partial, weak, or no  
similarity with any known sequence. Unexpectedly, the level of  
reiteration within this replicon is low, with only two genes  
duplicated with more than 90% nucleotide sequence identity,  
transposon elements accounting for 2.2% of the sequence, and a few  
hundred short repeated palindromic motifs (RIME1, RIME2, and C)  
widespread over the chromosome. Three regions with a significantly  
lower GC content are most likely of external origin. Detailed  
annotation revealed that this replicon contains all housekeeping  
genes except two essential genes that are located on pSymB. Amino  
acid/peptide transport and degradn. and sugar metab. appear as two  
major features of the *S. meliloti* chromosome. The presence in this  
replicon of a large no. of nucleotide cyclases with a peculiar  
structure, as well as of genes homologous to virulence determinants  
of animal and plant pathogens, opens perspectives in the study of  
this bacterium both as a free-living soil microorganism and as a  
plant symbiont.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L11 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2003 ACS

Searcher : Shears 308-4994

09/912697

ACCESSION NUMBER: 2000:666920 HCAPLUS  
DOCUMENT NUMBER: 133:248033  
TITLE: Mismatch repair detection utilizing bacteria to detect mismatches by a change in expression of a marker gene  
INVENTOR(S): Cox, David R.; Faham, Malek; Baharloo, Siamak  
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, USA; The Regents of the University of California  
SOURCE: PCT Int. Appl., 55 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 4  
PATENT INFORMATION:

| PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE     |
|---|------|----------|-----------------|----------|
| WO 2000055369   | A1   | 20000921 | WO 2000-US6731  | 20000314 |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM<br>RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG   |      |          |                 |          |
| US 6406847  | B1   | 20020618 | US 1999-271055  | 19990317 |
| EP 1175510  | A1   | 20020130 | EP 2000-916338  | 20000314 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO   |      |          |                 |          |
| JP 2002538840   | T2   | 20021119 | JP 2000-605785  | 20000314 |
| PRIORITY APPLN. INFO.:  |      |          |                 |          |
| US 1999-271055 A 19990317<br>US 1995-4664P P 19951002<br>US 1996-713751 B2 19960913<br>WO 2000-US6731 W 20000314  |      |          |                 |          |
| AB Mismatch Repair Detection (MRD), a novel method for DNA-variation detection, utilizes bacteria to detect mismatches by a change in expression of a marker gene. DNA fragments to be screened for variation are cloned into two MRD plasmids, and bacteria are transformed with heteroduplexes of these constructs. Resulting colonies express the marker gene in the absence of a mismatch, and lack expression in the presence of a mismatch. MRD is capable of detecting a single mismatch within 10 kb of DNA. In addn., MRD can analyze many fragments simultaneously, offering a powerful method for high-throughput genotyping and mutation detection. Mismatch Repair Detection (MRD), a novel method for DNA-variation detection, utilizes bacteria to detect mismatches by a change in expression of a marker gene. DNA fragments to be screened for variation are cloned into two MRD plasmids, and bacteria are transformed with heteroduplexes of these constructs. Resulting colonies express the marker gene in the absence and a mismatch, and lack expression in the presence of a mismatch. MRD is capable of detecting a single mismatch within 10 kb of DNA. In addn., MRD has the potential for analyzing many fragments simultaneously, offering a powerful method for high-throughput genotyping and mutation detection in a large genomic region. The method was demonstrated using two puC-derived |      |          |                 |          |

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plasmids, pMF200 and pMF100, which are identical except that there is a 5-bp insertion in the lacZ.alpha. gene of pMF100. The method of mutation detection comprises cloning one copy of the DNA in question in pMF200, the other copy into pMF100. The pMF200 plasmid is cloned in dam- Escherichia coli (no methylation of the plasmid); the pMF100 plasmid is cloned in dam+ E. coli (methylation of plasmid). The plasmids are isolated, linearized, denatured, and reannealed, then digested with MboI and DpnI. E. coli are transformed with the resulting hemimethylated heteroduplexes. The transformants are cultured and .beta.-galactosidase activity detected as usual. If no mutation was present (i.e., no mismatch), no repair occurs and the colonies are blue. If a mutation was present, repair occurs and the lacZ.alpha. mutant is corepaired resulting in colonies with white color.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1993:206476 HCAPLUS

DOCUMENT NUMBER: 118:206476

TITLE: Sequence and transcriptional analysis of the Streptomyces glaucescens tcmAR tetracenomycin C resistance and repressor gene loci

AUTHOR(S): Guilfoile, Patrick G.; Hutchinson, C. Richard  
CORPORATE SOURCE: Sch. Pharm., Univ. Wisconsin, Madison, WI, 53706, USA

SOURCE: Journal of Bacteriology (1992), 174(11), 3651-8  
CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Sequence anal. of the tcmA tetracenomycin C resistance gene from S. glaucescens GLA.O (ETH 22794) identifies one large open reading frame whose deduced product has sequence similarity to the **mmr** methylenomycin resistance gene from S. coelicolor, the S. rimosus tet347 (otrB) **tetracycline** resistance gene, and the atrI aminotriazole resistance gene from Saccharomyces cerevisiae. These genes are thought to encode proteins that act as metabolite export pumps powered by transmembrane electrochem. gradients. A divergently transcribed gene, tcmR, is located in the region upstream of tcmA. The deduced product of tcmR resembles the repressor proteins encoded by tetR regulatory genes from Escherichia coli and the actII-orf1 gene from S. coelicolor. Transcriptional anal. of tcmA and tcmR indicates that these genes have back-to-back and overlapping promoter regions.

L11 ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1992:169868 HCAPLUS

DOCUMENT NUMBER: 116:169868

TITLE: An integrated approach to studying regulation of production of the **antibiotic**

methylenomycin by Streptomyces coelicolor A3(2)  
AUTHOR(S): Hobbs, Glyn; Obanye, Anthony I. C.; Petty, June; Mason, J. Clark; Barratt, Elizabeth; Gardner, David C. J.; Flett, Fiona; Smith, Colin P.; Broda, Paul; Oliver, Stephen G.

CORPORATE SOURCE: Manchester Biotechnol. Cent., Univ. Manchester  
Inst. Sci. and Technol., Manchester, M60 1QD, UK

Searcher : Shears 308-4994

09/912697

SOURCE: Journal of Bacteriology (1992), 174(5), 1487-94  
CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A physiol. and mol. biol. study was made of the control of methylenomycin biosynthesis by *S. coelicolor* A3(2).. A simple and reliable assay for this **antibiotic** was developed. Conditions that permit the synthesis of methylenomycin by *S. coelicolor* cultures grown in defined medium were elucidated: a readily assimilated C and N source is required. Under these conditions, methylenomycin is produced late in the growth phase, at the time of transition from exponential to linear growth. Provided that the phosphate concn. in the medium is kept high, there is synthesis of methylenomycin but not of the other secondary metabolites that this strain can produce. These conditions were used to study the transcription of the methylenomycin gene cluster during the transition from primary to secondary metab. The biosynthetic genes of .gtoreq.1 of the mmy transcription units appear to be transcribed before the mmr resistance determinant. The possibility that methylenomycin induces the transcription of mmr is discussed.

L11 ANSWER 8 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1991:442803 HCAPLUS

DOCUMENT NUMBER: 115:42803

TITLE: Efflux-mediated antiseptic resistance gene qacA from *Staphylococcus aureus*: common ancestry with **tetracycline**- and sugar-transport proteins

AUTHOR(S): Rouch, D. A.; Cram, D. S.; DiBerardino, D.; Littlejohn, T. G.; Skurray, R. A.

CORPORATE SOURCE: Dep. Microbiol., Monash Univ., Clayton, 3168, Australia

SOURCE: Molecular Microbiology (1990), 4(12), 2051-62  
CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Resistance to intercalating dyes (ethidium, acriflavine) and other org. cations, such as quaternary ammonium-type antiseptic compds., mediated by the *S. aureus* plasmid pSK1 is specified by an energy-dependent export mechanism encoded by the qacA gene. From nucleotide sequence anal., qacA is predicted to encode a protein of Mr 55,017 contg. 514 amino acids. The gene is likely to initiate with a CUG codon, and a 36 bp palindrome immediately preceding qacA, along with an upstream reading frame ORF188 with homol. to the TetR repressors, may be components of a regulatory circuit. The putative polypeptide specified by qacA has properties typical of a cytoplasmic membrane protein, and is indicated to be a member of a transport protein family that includes proteins responsible for export-mediated resistance to **tetracycline** and methylenomycin, and uptake of sugars and quinate. The anal. suggests that N- and C-terminal regions of these proteins are involved in energy coupling (proton translocation) and substrate transport, resp. The last common ancestor of the qacA and related tet (**tetracycline** resistance) lineages is inferred to have been repressor controlled, as occurs for modern tet determinants from gram-neg., but not those from gram-pos., bacteria.

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L11 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1989:109164 HCAPLUS

DOCUMENT NUMBER: 110:109164

TITLE: Strand targeting signal(s) for in vivo mutation avoidance by post-replication mismatch repair in *Escherichia coli*

AUTHOR(S): Claverys, Jean Pierre; Mejean, Vincent

CORPORATE SOURCE: Cent. Rech. Biochim. Genet. Cell., Univ. Paul Sabatier, Toulouse, F-31062, Fr.

SOURCE: Molecular and General Genetics (1988), 214(3), 574-8

CODEN: MGGEAE; ISSN: 0026-8925

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The involvement of GATC sites in directing mismatch correction for the elimination of replication errors in *E. coli* was investigated in vivo by analyzing mutation rates for a gene carried on a series of related plasmids that contain 2, 1 and 0 such sites. This gene encoding **chloramphenicol** acetyltransferase (Cat protein) was inactivated by a point mutation. In vivo mutations restoring resistance to **chloramphenicol** were scored in mismatch repair proficient (mut+) and deficient (mutHLS-) strains. In mut+ cells, redn. of GATC sites from 2 to 0 increased mutation rates approx. 10-fold. Removal of the GATC site distal to the cat-mutation increased the rate of mutation less than 2-fold, indicating that mismatch repair can proceed normally with a single site. The mutation rate increased 3-fold after removal of the GATC site proximal to the mutation. In the absence of a GATC site, mutL- and mutS- strains exhibited a 2- to 3-fold increased mutation rate as compared to isogenic mutH- and mut+ strains. This indicates that 50%-70% of replication errors can be cor. in a mutLS-dependent way in the absence of any GATC site to target mismatch correction to newly synthesized DNA strands. Other strand targeting signals, possibly single strand discontinuities, might be used in mutLS-dependent repair.

L11 ANSWER 10 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1988:181052 HCAPLUS

DOCUMENT NUMBER: 108:181052

TITLE: Nucleotide sequence analysis reveals similarities between proteins determining methylenomycin A resistance in *Streptomyces* and **tetracycline** resistance in eubacteria

AUTHOR(S): Neal, Robert J.; Chater, Keith F.

CORPORATE SOURCE: John Innes Inst., Norwich, NR4 7UH, UK

SOURCE: Gene (1987), 58(2-3), 229-41

CODEN: GENED6; ISSN: 0378-1119

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Previous studies had localized the **gene** (mmr) for resistance to methylenomycin A (Mm) to a 2.5-kb PstI fragment in the middle of a cluster of Mm biosynthetic genes from the *S. coelicolor* plasmid SCP1. In this paper, the gene has been more precisely located by sub-cloning, and the nucleotide sequence of the whole fragment has been detd. The predicted mmr-specified protein (Mr 49,238) would be hydrophobic, with some homol. at the amino acid level to **tetracycline**-resistance proteins from both gram-pos. and gram-neg. bacteria. Comparisons of hydropathy plots

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of the amino acid sequences reinforces the idea that the proteins are similar. It is suggested that Mm resistance may be conferred by a membrane protein, perhaps controlling efflux of the **antibiotic**. No significant homol. was detected by hybridization anal. between **mmr** and a cloned oxytetracycline (OTc)-resistance gene (**tetB**) of the OTc producer *S. rimosus*, and no cross-resistance was conferred by these genes. Sequences on both sides of **mmr** appear to encode proteins. The direction of translation in each case would be opposite to that of **mmr** translation. This suggests that **mmr** is transcribed as a monocistronic mRNA from a bidirectional promoter. An extensive inverted repeat sequence between the stop codons of **mmr** and the converging **gene** may function as a bidirectional transcription terminator.

IT **60-54-8, Tetracycline**

RL: PRP (Properties)

(gene for resistance to, of eubacteria, *Streptomyces* methylenomycin A resistance gene in relation to)

L11 ANSWER 11 OF 11 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1987:471849 HCAPLUS

DOCUMENT NUMBER: 107:71849

TITLE: Comparison of the rep-38 and **mmrA1** mutations of *Escherichia coli*

AUTHOR(S): Sharma, Rakesh C.; Smith, Kendric C.

CORPORATE SOURCE: Sch. Med., Stanford Univ., Stanford, CA, 94305, USA

SOURCE: Mutation Research (1987), 184(1), 23-8

CODEN: MUREAV; ISSN: 0027-5107

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The rep-38 and **mmrA1** mutations are located very close to each other (.apprx.85 min), and have been suggested to be allelic. To address this question, the phenotypes of the **mmrA1** and rep-38 mutants were compared. Both the **mmrA1** and rep-38 mutations blocked the enhanced killing and inhibition of postreplication repair by rich growth medium that occurs in UV-irradiated *E. coli* K-12 **uvrA** cells, i.e., the **mmrA1** and rep-38 strains did not show minimal medium recovery (MMR). However, **.vphi.174** bacteriophage propagated well in **mmrA1** strains, but not in rep-38 strains; a rep mutation sensitized a **wvrA** strain to UV irradiation, but a **mmrA** mutation did not. During **chloramphenicol** treatment, the rep-38 strain showed a larger amt. of residual DNA synthesis than obsd. in the **mmrA1** strain. The **mmrA1** mutation appears to be a dominant mutation. This was detd. by the failure of either plasmid pLC44-7 or episome F'KLF11, both of which carry the **mmrA+** **gene**, to complement the **Mmr** - phenotype of a **uvrA mmrA** strain. Plasmid pLC44-7 is known to complement the rep-38 mutation, suggesting that rep-38 is a recessive mutation. Although certain of the phenotypes of the rep and **mmrA** mutants are similar, a no. are quite different. These differences suggest that these 2 mutations are not allelic.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 14:38:05 ON 06 JAN 2003)

L12 23 S L10

L13 15 DUP REM L12 (8 DUPLICATES REMOVED)

L13 ANSWER 1 OF 15 WPIDS (C) 2003 THOMSON DERWENT

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ACCESSION NUMBER: 2002-599624 [64] WPIDS  
DOC. NO. NON-CPI: N2002-475437  
DOC. NO. CPI: C2002-169445  
TITLE: Making hypermutable cell for agricultural,  
pharmaceutical or environmental applications, by  
exposing cell to mismatch repair inhibitor such as  
anthracene, ATPase inhibitor, nuclease inhibitor or  
polymerase inhibitor.  
DERWENT CLASS: B04 B05 C03 D16 P13  
INVENTOR(S): GRASSO, L; NICOLAIDES, N C; SASS, P M  
PATENT ASSIGNEE(S): (MORP-N) MORPHOTEK INC  
COUNTRY COUNT: 94  
PATENT INFORMATION:

| PATENT NO   | KIND | DATE     | WEEK      | LA | PG  |
|---|------|----------|-----------|----|-----|
| WO 2002054856   | A1   | 20020718 | (200264)* | EN | 114 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC |      |          |           |    |     |
| MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW                          |      |          |           |    |     |
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE  |      |          |           |    |     |
| DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG     |      |          |           |    |     |
| KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ     |      |          |           |    |     |
| PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN     |      |          |           |    |     |
| YU ZA ZW  |      |          |           |    |     |

APPLICATION DETAILS:

| PATENT NO     | KIND | APPLICATION   | DATE     |
|---------------|------|---------------|----------|
| WO 2002054856 | A1   | WO 2001-US934 | 20010115 |

PRIORITY APPLN. INFO: WO 2001-US934 20010115

AN 2002-599624 [64] WPIDS

AB WO 200254856 A UPAB: 20021007

NOVELTY - Making (M1) a hypermutable cell, comprising exposing a cell to an inhibitor of mismatch repair (MMR), where the inhibitor is an anthracene, an ATPase inhibitor, a nuclease inhibitor, a polymerase inhibitor, or an antisense oligonucleotide that specifically hybridizes to a nucleotide encoding a MMR protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) generating (M2) a mutation in a gene of interest, comprising:

(a) exposing a cell or an animal comprising the **gene** of interest to a chemical **MMR** inhibitor and testing the cell or animal to determine if the gene of interest comprises a mutation; or

(b) growing a plant comprising the gene of interest, exposing the plant to an inhibitor of MMR, and testing the plant to determine if the gene of interest comprises a mutation;

(2) a hypermutable transgenic mammal (I) made by M2;

(3) generating (M3) a MMR defective plant by exposing the plant to an inhibitor of MMR;

(4) a hypermutable plant (II) made by M3;

(5) screening (M4) for chemical inhibitor of MMR by exposing an organism to a candidate compound and screening the DNA of the



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organism for microsatellite instability; and

(6) blocking (M5) MMR activity in vivo by exposing a cell to an anthracene compound.

USE - M1 is useful for making a hypermutable cell. M2 is useful for generating a mutation in a gene of interest. M3 is useful for generating a MMR defective plant. M4 is useful for screening for chemical inhibitor of MMR. M5 is useful for blocking MMR activity in vivo. (All claimed). M1 is useful for creating genetically altered host cells or organisms for agricultural, chemical manufacturing, pharmaceutical and environmental applications.

ADVANTAGE - Several advantages exist in generating genetic mutations by blocking MMR in vivo in contrast to general DNA damaging agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methylnitrosourea (MNU) and ethyl methanesulfonate (EMS). Cells with MMR deficiency have a wide range of mutations dispersed throughout their entire genome in contrast to DNA damaging agents such as MNNG, MNU and EMS and ionizing radiation. Another advantage is that mutant cells that arise from MMR deficiency are diploid in nature and do not lose large segments of chromosomes as is the case of DNA damaging agents such as EMS, MNU, and ionizing radiation. This unique feature allows for subtle changes throughout a host's genome that leads to subtle genetic changes yielding genetically stable hosts with commercially important output traits.

Dwg.0/8

L13 ANSWER 2 OF 15 WPIDS (C) 2003 THOMSON DERWENT

ACCESSION NUMBER: 2001-522820 [57] WPIDS

DOC. NO. CPI: C2001-156138

TITLE: Making hypermutable yeast that exhibit novel selected output traits for commercial applications, comprises introducing polynucleotide containing dominant negative allele of **mismatch repair gene**.

DERWENT CLASS: B04 D16

INVENTOR(S): GRASSO, L; KINZLER, K W; NICOLAIDES, N C; SASS, P M; VOGELSTEIN, B; ALIS, J M

PATENT ASSIGNEE(S): (GRAS-I) GRASSO L; (KINZ-I) KINZLER K W; (NICO-I) NICOLAIDES N C; (SASS-I) SASS P M; (UYJO) UNIV JOHNS HOPKINS; (VOGE-I) VOGELSTEIN B; (ALIS-I) ALIS J M

COUNTRY COUNT: 94

PATENT INFORMATION:

| PATENT NO     | KIND | DATE     | WEEK      | LA | PG |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |
|---------------|------|----------|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--|
| WO 2001062945 | A1   | 20010830 | (200157)* | EN | 59 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |
| RW:           | AT   | BE       | CH        | CY | DE | DK | EA | ES | FI | FR | GB | GH | GM | GR | IE | IT | KE | LS | LU | MC |  |
|               | MW   | MZ       | NL        | OA | PT | SD | SE | SL | SZ | TR | TZ | UG | ZW |    |    |    |    |    |    |    |  |
| W:            | AE   | AG       | AL        | AM | AT | AU | AZ | BA | BB | BG | BR | BY | BZ | CA | CH | CN | CR | CU | CZ | DE |  |
|               | DK   | DM       | DZ        | EE | ES | FI | GB | GD | GE | GH | GM | HR | HU | ID | IL | IN | IS | JP | KE | KG |  |
|               | KP   | KR       | KZ        | LC | LK | LR | LS | LT | LU | LV | MA | MD | MG | MK | MN | MW | MX | MZ | NO | NZ |  |
|               | PL   | PT       | RO        | RU | SD | SE | SG | SI | SK | SL | TJ | TM | TR | TT | TZ | UA | UG | US | UZ | VN |  |
|               | YU   | ZA       | ZW        |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |
| AU 2001038558 | A    | 20010903 | (200202)  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |
| US 2002123149 | A1   | 20020905 | (200260)  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |
| US 6454146    | B2   | 20020924 | (200266)  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |

APPLICATION DETAILS:

Searcher : Shears 308-4994

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| PATENT NO     | KIND           | APPLICATION     | DATE     |
|---------------|----------------|-----------------|----------|
| WO 2001062945 | A1             | WO 2001-US5447  | 20010221 |
| AU 2001038558 | A              | AU 2001-38558   | 20010221 |
| US 2002123149 | A1 Provisional | US 2000-184336P | 20000223 |
|               |                | US 2001-788657  | 20010221 |
| US 6454146    | B2 Provisional | US 2000-184336P | 20000223 |
|               |                | US 2001-770348  | 20010126 |

FILING DETAILS:

| PATENT NO     | KIND       | PATENT NO    |
|---------------|------------|--------------|
| AU 2001038558 | A Based on | WO 200162945 |

PRIORITY APPLN. INFO: US 2000-184336P 20000223; US 2001-788657  
20010221; US 2001-770348 20010126

AN 2001-522820 [57] WPIDS

AB WO 200162945 A UPAB: 20011005

NOVELTY - Making (M1) a hypermutable yeast (I), comprising introducing a polynucleotide (II) containing a dominant negative allele (III) of a **mismatch repair (MMR)** gene, into a yeast, whereby the cell becomes hypermutable, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a homogeneous composition (HC) of cultured, hypermutable, yeast comprising (III);

(2) generating (M2) a mutation in a gene of interest (GI) comprising:

(a) growing a yeast culture containing GI and (III), where the cell is hypermutable, and testing the cell to determine whether GI harbors a mutation; or

(b) growing a yeast cell containing GI and a polynucleotide encoding (III), to create a population of mutated, hypermutable yeast cells, cultivating the population under trait selection conditions, and testing at least one of the cultivated yeast cell to determine whether GI harbors a mutation;

(3) generating (M3) enhanced hypermutable yeast comprising exposing a yeast cell containing (III) to a mutagen, such that an enhanced rate of mutation of the yeast cell is achieved; and

(4) generating (M4) an MMR-proficient yeast with a new output traits comprising growing a MMR-deficient yeast cell containing GI and a polynucleotide encoding (III), to create a population of mutated, hypermutable yeast, cultivating the yeast population under trait selection conditions, testing the yeast cells to determine that GI harbors a mutation, and restoring MMR activity to the yeast cells.

USE - The method is useful to create desirable output traits for commercial applications, using dominant negative alleles of mismatch repair proteins. (I) is useful for production, biocatalysis, bioremediation and drug discovery. (I) is useful in genetic screens for the direct selection of variant subclones that exhibit new output traits. (I) is also useful in manufacturing industry for the generation of new biochemicals, for detoxifying noxious chemicals from by-products of manufacturing processes or those used as catalysts, for remediation of toxins present in the

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environment including polychlorobenzenes, heavy metals and other environmental hazards for which there is a need to remove them from the environment. (I) is also useful for screening novel mutations in a gene or a set of genes that produce variant siblings that exhibit a new output trait not found in wild type cells. The yeast is also useful for producing increased quantity or quality of protein or non-protein therapeutic molecule e.g., Penicillin G, Erythromycin and Clavulanic acid, by biotransformation. (III) is useful for producing higher quantities of a recombinant polypeptides.

ADVANTAGE - (I) has increased performance characteristics and abilities. The use of (I) in genetic screens bypass the tedious and time-consuming steps of gene identification, isolation and characterization. The yeast strain display novel output features that are suitable for a wide variety of applications.

Dwg.0/0

L13 ANSWER 3 OF 15 WPIDS (C) 2003 THOMSON DERWENT  
ACCESSION NUMBER: 2001-514664 [56] WPIDS  
DOC. NO. CPI: C2001-153855  
TITLE: Making hypermutable bacteria for biocatalysis, bioremediation and drug discovery, involves introducing polynucleotide comprising dominant negative allele of **mismatch repair gene** under regulatory sequence control.  
DERWENT CLASS: B04 D16  
INVENTOR(S): GRASSO, L; KINZLER, K W; NICOLAIDES, N C; SASS, P M; VOGELSTEIN, B  
PATENT ASSIGNEE(S): (UYJO) UNIV JOHNS HOPKINS; (GRAS-I) GRASSO L; (KINZ-I) KINZLER K W; (NICO-I) NICOLAIDES N C; (SASS-I) SASS P M; (VOGE-I) VOGELSTEIN B  
COUNTRY COUNT: 94  
PATENT INFORMATION:

| PATENT NO   | KIND       | DATE | WEEK               | LA | PG |
|---|------------|------|--------------------|----|----|
| WO  | 2001059092 | A2   | 20010816 (200156)* | EN | 68 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC |            |      |                    |    |    |
| MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW                          |            |      |                    |    |    |
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE  |            |      |                    |    |    |
| DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG     |            |      |                    |    |    |
| KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ     |            |      |                    |    |    |
| PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN     |            |      |                    |    |    |
| YU ZA ZW  |            |      |                    |    |    |
| AU  | 2001034992 | A    | 20010820 (200175)  |    |    |
| US  | 2002068284 | A1   | 20020606 (200241)  |    |    |

APPLICATION DETAILS:

| PATENT NO | KIND       | APPLICATION    | DATE                     |
|-----------|------------|----------------|--------------------------|
| WO        | 2001059092 | A2             | WO 2001-US4339 20010212  |
| AU        | 2001034992 | A              | AU 2001-34992 20010212   |
| US        | 2002068284 | A1 Provisional | US 2000-181929P 20000211 |
|           |            |                | US 2001-780675 20010212  |

FILING DETAILS:

Searcher : Shears 308-4994

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| PATENT NO     | KIND | PATENT NO |
|---------------|------|-----------|
| AU 2001034992 | A    | Based on  |
| WO 200159092  |      |           |

PRIORITY APPLN. INFO: US 2000-181929P 20000211; US 2001-780675  
20010212

AN 2001-514664 [56] WPIDS

AB WO 200159092 A UPAB: 20011001

NOVELTY - Making (M1) a hypermutable bacteria (I), comprising introducing a polynucleotide (II) having a dominant negative allele (III) of a **mismatch repair (MMR) gene** under the control of an inducible transcription regulatory sequence, into a bacterium, is new. The cell becomes inducibly hypermutable.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a homogeneous composition (HC) of cultured, hypermutable, bacteria comprising (III);

(2) generating (M2) a mutation in a gene of interest (GI), by:

(a) growing bacterial culture comprising GI and (III), where the cell is hypermutable, and testing the cell to determine if GI harbors a mutation; or

(b) growing a bacterium comprising GI and (III), to form a population of mutated bacteria, cultivating the population under trait selection conditions, and testing at least one of the cultivated bacteria to determine whether GI harbors a mutation;

(3) enhancing (M3) the mutation rate of a bacterium, by exposing a bacterium comprising (III) to a mutagen, the mutation rate of the bacterium is enhanced in excess of the rate in the absence of mutagen or (III); and

(4) generating (M4) an MMR-proficient bacterium with a new output trait, by growing a MMR-deficient bacterium comprising a defective **MMR gene** allele and GI, to form a population of mutated bacteria, cultivating the bacterial population under trait selection conditions, testing at least one of the cultivated bacteria to determine that GI harbors a mutation, and restoring MMR activity to at least one cultivated bacteria.

USE - The method is useful to create desirable output traits for commercial applications, using dominant negative alleles of mismatch repair proteins. (I) is useful for production, biocatalysis, bioremediation and drug discovery. (I) is also useful in manufacturing industry for the generation of new biochemicals useful for detoxifying noxious chemicals from by-products of manufacturing processes or those used as catalysts, for remediation of toxins present in the environment including polychlorobenzenes, heavy metals and other environmental hazards for which there is a need to remove them from the environment. (I) is also useful for screening novel mutations in a gene or a set of genes that produce variant siblings that exhibit a new output trait not found in wild type cells. The bacteria are also useful for producing increased quantity or quality of protein or non-protein therapeutic molecule e.g. Penicillin G, Erythromycin and Clavulanic acid, by biotransformation. (III) is useful for producing higher quantities of a recombinant polypeptides.

Dwg.0/6

L13 ANSWER 4 OF 15 MEDLINE  
ACCESSION NUMBER: 2001541278 MEDLINE

Searcher : Shears 308-4994

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DOCUMENT NUMBER: 21472261 PubMed ID: 11587853  
TITLE: Transcriptional regulation of the **mismatch repair gene hMLH1**.  
AUTHOR: Quaresima B; Faniello M C; Baudi F; Cuda G; Grandinetti C; Tassone P; Costanzo F; Venuta S  
CORPORATE SOURCE: Dipartimento di Medicina Sperimentale e Clinica G. Salvatore, Universita degli Studi di Catanzaro Magna Graecia, via T. Campanella 115, 88100 Catanzaro, Italy.  
SOURCE: GENE, (2001 Sep 19) 275 (2) 261-5.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200112  
ENTRY DATE: Entered STN: 20011008  
Last Updated on STN: 20020122  
Entered Medline: 20011207

AB We have characterized the promoter region of the human **gene** coding for the MLH1 **mismatch repair** protein. The total transcriptional activity of the hMLH1 promoter is driven by two positive cis-elements included between nucleotides -300 and -220. The upstream element is a canonical CCAAT box, and it is recognized by the heterotrimeric transcription factor NF-Y. On the other hand, the downstream element is recognized by a nuclear factor of about 120 kDa. Variations in hMLH1 intracellular levels may influence the surveillance of the genome integrity. The identification of the two elements may shed some light on the regulation of the transcriptional regulation of hMLH1 expression.

L13 ANSWER 5 OF 15 JICST-EPlus COPYRIGHT 2003 JST

ACCESSION NUMBER: 991026844 JICST-EPlus  
TITLE: Differential cytotoxicity of anticancer agents in hMutS.ALPHA.-deficient and -proficient human colorectal cancer cells.  
AUTHOR: UCHIDA I; ZHONG X  
CORPORATE SOURCE: Toho Univ. School Of Medicine, Tokyo, Jpn  
SOURCE: Soshiki Baiyo Kenkyu (Tissue Culture Research Communications), (1999) vol. 18, no. 3, pp. 301-312.  
Journal Code: Z0362B (Fig. 6, Tbl. 1, Ref. 52)  
ISSN: 0912-3636  
PUB. COUNTRY: Japan  
DOCUMENT TYPE: Journal; Article  
LANGUAGE: English  
STATUS: New

AB Mismatch repair (MMR)-deficient cells exhibit drug resistance to several anticancer agents including N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), cisplatin, and adriamycin. Since these agents are potent mutagens, it is possible to select resistant clones of tumor cells during chemotherapy. Prior to determining whether drug cytotoxicity was altered by MMR-deficiency, mutation in the (A)8 repeat region of the hMSH3 **gene** of the **MMR**-deficient human colorectal cancer cell line HCT116 and the MMR-proficient human chromosome 3-transferred HCT116 (HCT116+ch3) was confirmed. A screening method was then determined using MNNG cytotoxicity in both cell lines and 20 additional anticancer agents were examined. Clonogenic cytotoxic assay revealed

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in 8 anticancer agents (streptozotocin, 5-fluorouracil, tegafur, bleomycin, mitomycin C, vinblastine, vincristine, and nitoran) maintaining the desired level of cytotoxicity required a higher concentration in HCT116 than in HCT116+ch3. Cytosine .BETA.-D-arabinofuranoside, chlorambucil, and epirubicin were more cytotoxic to HCT116. Dacarbazine, nitrogen mustard, 3'-azido-3'-deoxythymidine, aclarubicin, neocarzinostatin, actinomycin D, and peplomycin possessed similar cytotoxicity. These results suggest that drugs with higher or uncompromised sensitivity can circumvent drug resistance due to MMR-deficiency in tumor cells. (author abst.)

L13 ANSWER 6 OF 15 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998381272 EMBASE

TITLE: **mmr**, a Mycobacterium tuberculosis gene conferring resistance to small cationic dyes and inhibitors.

AUTHOR: De Rossi E.; Branzoni M.; Cantoni R.; Milano A.; Riccardi G.; Ciferri O.

CORPORATE SOURCE: O. Ciferri, Dept. of Genetics and Microbiology, via Abbiategrasso 207, 27100 Pavia, Italy. ociferri@pillo.unipv.it

SOURCE: Journal of Bacteriology, (1998) 180/22 (6068-6071). Refs: 24

ISSN: 0021-9193 CODEN: JOBAAY

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology  
037 Drug Literature. Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The **mmr** gene, cloned from Mycobacterium tuberculosis, was shown to confer to Mycobacterium smegmatis resistance to tetraphenylphosphonium (TPP), erythromycin, ethidium bromide, acriflavine, safranin O, and pyronin Y. The gene appears to code for a protein containing four transmembrane domains. Studies of [3H]TPP intracellular accumulation strongly suggest that the resistance mediated by the Mmr protein involves active extrusion of TPP.

L13 ANSWER 7 OF 15 MEDLINE

ACCESSION NUMBER: 97433116 MEDLINE

DOCUMENT NUMBER: 97433116 PubMed ID: 9288785

TITLE: Mutator phenotype in Msh2-deficient murine embryonic fibroblasts.

AUTHOR: Reitmair A H; Risley R; Bristow R G; Wilson T; Ganesh A; Jang A; Peacock J; Benchimol S; Hill R P; Mak T W; Fishel R; Meuth M

CORPORATE SOURCE: Ontario Cancer Institute/Amgen Institute, Department of Medical Biophysics, University of Toronto, Canada.

CONTRACT NUMBER: R01 CA22188 (NCI)

R01 CA56542 (NCI)

R01 CA62244 (NCI)

+

SOURCE: CANCER RESEARCH, (1997 Sep 1) 57 (17) 3765-71. Journal code: 2984705R. ISSN: 0008-5472.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

Searcher : Shears 308-4994

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LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199709  
ENTRY DATE: Entered STN: 19971008  
Last Updated on STN: 19990129  
Entered Medline: 19970924

AB Embryonic fibroblast cell lines were established from mice deficient, heterozygous, or proficient for Msh2, one of the three known DNA mismatch repair genes involved in hereditary nonpolyposis colon cancer (HNPCC). Cell lines were established by transfection of primary mouse embryo fibroblasts with E7 and Ras oncogenes or mutant p53. Spontaneously immortalized cells derived from the primary cultures were also studied. To determine whether these cells developed a mutator phenotype similar to that found in colon cancer cells deficient in mismatch repair, we measured mutation rates, microsatellite instability, and sensitivities to a range of DNA-damaging agents. The mutator phenotype detected in the E7 and Ras or mutant p53-immortalized Msh2<sup>-/-</sup> mouse cells was similar to that found in human mismatch repair-deficient colorectal carcinoma cell lines. Mutation rates to ouabain resistance were increased 8-12-fold relative to lines from Msh2<sup>+/+</sup> mice, and microsatellite instability was detectable in 12-18% of subclones derived from the Msh2<sup>-/-</sup> line but was undetectable in subclones developed from the Msh2<sup>+/+</sup> line. Furthermore, E7 and Ras or spontaneously immortalized Msh2<sup>-/-</sup> cells were significantly more resistant to the cytotoxic effects of 6-thioguanine relative to Msh2<sup>+/+</sup> cells. In contrast, these lines showed various responses to UV light and cis-platinum, suggesting that mismatch repair deficiency was not the sole determinant for sensitivity to these DNA-damaging agents. Particular attention was paid to the properties of cells heterozygous for the Msh2 mutant gene, which would mimic the situation of an HNPCC carrier. However, our studies failed to reveal any properties of these cells that might provide a growth advantage or predispose them for the acquisition of further mutations. This observation is consistent with the model that inactivation of the wild-type Msh2 allele is a critical step for tumorigenesis in HNPCC patients.

L13 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 1993:499908 BIOSIS  
DOCUMENT NUMBER: PREV199396123915  
TITLE: Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair.  
AUTHOR(S): Strand, Micheline (1); Prolla, Tomas A.; Liskay, R. Michael; Petes, Thomas D.  
CORPORATE SOURCE: (1) Dep. Biol.; Univ. North Carolina, Chapel Hill, NC 27599-3280 USA  
SOURCE: Nature (London), (1993) Vol. 365, No. 6443, pp. 274-276.  
ISSN: 0028-0836.  
DOCUMENT TYPE: Article  
LANGUAGE: English

AB THE genomes of all eukaryotes contain tracts of DNA in which a single base or a small number of bases is repeated. Expansions of such tracts have been associated with several human disorders including the fragile X syndrome-1. In addition, simple repeats are unstable in certain forms of colorectal cancer, suggesting a defect in DNA replication or repair-2-4. We show here that mutations in any

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three yeast **genes** involved in DNA **mismatch repair** (PMS1, MLH1 and MSH2) lead to 100- to 700-fold increases in tract instability, whereas mutations that eliminate the proof-reading function of DNA polymerases have little effect. The meiotic stability of the tracts is similar to the mitotic stability. These results suggest that tract instability is associated with DNA polymerases slipping during replication, and that some types of colorectal cancer may reflect mutations in **genes** involved in DNA **mismatch repair**.

L13 ANSWER 9 OF 15 MEDLINE DUPLICATE 1  
ACCESSION NUMBER: 92276347 MEDLINE  
DOCUMENT NUMBER: 92276347 PubMed ID: 1592819  
TITLE: Sequence and transcriptional analysis of the  
Streptomyces glaucescens tcmAR tetracenomycin C  
resistance and repressor gene loci.  
AUTHOR: Guilfoile P G; Hutchinson C R  
CORPORATE SOURCE: School of Pharmacy, University of Wisconsin, Madison  
53706.  
CONTRACT NUMBER: CA35381 (NCI)  
T32-GM07215 (NIGMS)  
SOURCE: JOURNAL OF BACTERIOLOGY, (1992 Jun) 174 (11) 3651-8.  
Journal code: 2985120R. ISSN: 0021-9193.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE).  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-M79367; GENBANK-M79368; GENBANK-M79369;  
GENBANK-M79370; GENBANK-M79371; GENBANK-M79372;  
GENBANK-M80674; GENBANK-M84973; GENBANK-M84974;  
GENBANK-M84980  
ENTRY MONTH: 199207  
ENTRY DATE: Entered STN: 19920710  
Last Updated on STN: 19950206  
Entered Medline: 19920701  
AB Sequence analysis of the tcmA tetracenomycin C resistance gene from  
Streptomyces glaucescens GLA.O (ETH 22794) identifies one large open  
reading frame whose deduced product has sequence similarity to the  
**mmr** methylenomycin resistance **gene** from  
Streptomyces coelicolor, the Streptomyces rimosus tet347 (otrB)  
**tetracycline** resistance gene, and the atr1 aminotriazole  
resistance gene from Saccharomyces cerevisiae. These genes are  
thought to encode proteins that act as metabolite export pumps  
powered by transmembrane electrochemical gradients. A divergently  
transcribed gene, tcmR, is located in the region upstream of tcmA.  
The deduced product of tcmR resembles the repressor proteins encoded  
by tetR regulatory genes from Escherichia coli and the actII-orf1  
gene from S. coelicolor. Transcriptional analysis of tcmA and tcmR  
indicates that these genes have back-to-back and overlapping  
promoter regions.

L13 ANSWER 10 OF 15 MEDLINE DUPLICATE 2  
ACCESSION NUMBER: 91276264 MEDLINE  
DOCUMENT NUMBER: 91276264 PubMed ID: 2055482  
TITLE: Bidirectional promoter and terminator regions bracket  
**mmr**, a resistance **gene** embedded in  
the Streptomyces coelicolor A3(2) gene cluster  
encoding methylenomycin production.

Searcher : Shears 308-4994



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AUTHOR: Neal R J; Chater K F  
CORPORATE SOURCE: John Innes Institute, John Innes Centre for Plant  
Science Research, Norwich, U.K.  
SOURCE: GENE, (1991 Apr) 100 75-83.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199107  
ENTRY DATE: Entered STN: 19910818  
Last Updated on STN: 19970203  
Entered Medline: 19910730

AB Low- and high-resolution nuclease mapping of in vivo transcripts, and in vitro transcription reactions using purified RNA polymerase, were used to analyse transcription of and around the **mmr** gene, which specifies resistance of *Streptomyces coelicolor* A3(2) to methylenomycin (Mm) and is located in the middle of a cluster of Mm-production-encoding genes. Transcription of **mmr** is from a single major start point (tsp) which is separated by only 81 bp from a divergent tsp. A pattern of direct and inverted repeats in the nucleotide sequence in this region may play a part in regulation of these promoters. The 3' end of the **mmr** transcript overlaps by 20-30 bp the 3' end of an RNA molecule involved in Mm production. The converging transcripts both terminate at the same large inverted repeat in the DNA. Purified RNA polymerase terminated transcription at this sequence in vitro (albeit only in one orientation).

L13 ANSWER 11 OF 15 MEDLINE

ACCESSION NUMBER: 89384567 MEDLINE  
DOCUMENT NUMBER: 89384567 PubMed ID: 2674679  
TITLE: Dual bidirectional promoters at the mouse dhfr locus: cloning and characterization of two mRNA classes of the divergently transcribed Rep-1 gene.  
AUTHOR: Linton J P; Yen J Y; Selby E; Chen Z; Chinsky J M; Liu K; Kellems R E; Crouse G F  
CORPORATE SOURCE: Department of Biology, Emory University, Atlanta, Georgia 30322.  
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1989 Jul) 9 (7) 3058-72.  
Journal code: 8109087. ISSN: 0270-7306.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-J04244; GENBANK-M24918; GENBANK-M24919  
ENTRY MONTH: 198910  
ENTRY DATE: Entered STN: 19900309  
Last Updated on STN: 19980206  
Entered Medline: 19891013

AB The mouse dihydrofolate reductase gene (dhfr) is a housekeeping gene expressed under the control of a promoter region embedded in a CpG island--a region rich in unmethylated CpG dinucleotides. A divergent transcription unit exists immediately upstream of the dhfr gene which is coamplified with dhfr in some but not all methotrexate-resistant cell lines. We show that the promoter region for this gene pair consists of two bidirectional promoters, a major

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and minor promoter, which are situated within a 660-base-pair region upstream of the dhfr ATG translation initiation codon. The major promoter controls over 90% of dhfr transcription, while the minor promoter directs the transcription of the remaining dhfr mRNAs. The major promoter functions bidirectionally, transcribing a divergent 4.0-kilobase poly(A) mRNA (class A) in the direction opposite that of dhfr transcription. The predicted protein product of this mRNA is 105 kilodaltons. The minor promoter also functions bidirectionally, directing the transcription of at least two divergent RNAs (class B). These RNAs, present in quantities approximately 1/10 to 1/50 that of the class A mRNAs, are 4.4- and 1.6-kilobase poly(A) mRNAs. cDNAs representing both class A and class B mRNAs have been cloned from a mouse fibroblast cell line which has amplified the dhfr locus (3T3R500). DNA sequence analysis of these cDNAs reveals that the class A and class B mRNAs share, for the most part, the same exons. On the basis of S1 nuclease protection analysis of RNA preparations from several mouse tissues, both dhfr and divergent genes showed similar levels of expression but did show some specificity in start site utilization. Computer homology searches have revealed sequence similarity of the divergent transcripts with bacterial **genes** involved in DNA **mismatch repair**, and we therefore have named the divergently transcribed gene Rep-1.

L13 ANSWER 12 OF 15 MEDLINE

ACCESSION NUMBER: 88040450 MEDLINE  
DOCUMENT NUMBER: 88040450 PubMed ID: 3313278  
TITLE: Mutation spectrum in Escherichia coli DNA mismatch repair deficient (mutH) strain.  
AUTHOR: Rewinski C; Marinus M G  
CORPORATE SOURCE: Department of Pharmacology, University of Massachusetts Medical School, Worcester 01655-2937.  
CONTRACT NUMBER: GM33233 (NIGMS)  
SOURCE: NUCLEIC ACIDS RESEARCH, (1987 Oct 26) 15 (20) 8205-15.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198712  
ENTRY DATE: Entered STN: 19900305  
Last Updated on STN: 19970203  
Entered Medline: 19871217

AB The Dam-directed post-replicative mismatch repair system of Escherichia coli removes base pair mismatches from DNA. The products of the mutH, mutL and mutS genes, among others, are required for efficient **mismatch repair**. Absence of any of these **gene** products leads to persistence of mismatches in DNA with a resultant increase in spontaneous mutation rate. To determine the specificity of the mismatch repair system in vivo we have isolated and characterized 47 independent mutations from a mutH strain in the plasmid borne mnt repressor gene. The major class of mutations comprises AT to GC transitions that occur within six base pairs of the only two 5'-GATC-3' sequences in the mnt gene. In the wild type control strain, insertion of the IS1 element was the major spontaneous mutational event. A prediction of the Dam-directed mismatch repair model, that the mutation spectra of dam and mutH strains should be the same, was confirmed.

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L13 ANSWER 13 OF 15 MEDLINE DUPLICATE 3  
ACCESSION NUMBER: 88112873 MEDLINE  
DOCUMENT NUMBER: 88112873 PubMed ID: 2828187  
TITLE: Nucleotide sequence analysis reveals similarities  
between proteins determining methylenomycin A  
resistance in Streptomyces and **tetracycline**  
resistance in eubacteria.  
AUTHOR: Neal R J; Chater K F  
CORPORATE SOURCE: John Innes Institute, Norwich, U.K.  
SOURCE: GENE, (1987) 58 (2-3) 229-41.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-M18263  
ENTRY MONTH: 198802  
ENTRY DATE: Entered STN: 19900305  
Last Updated on STN: 19900305  
Entered Medline: 19880226

AB Previous studies had localised the **gene (mmr)**  
for resistance to methylenomycin A (Mm) to a 2.5-kb PstI fragment in  
the middle of a cluster of Mm biosynthetic genes from the  
Streptomyces coelicolor plasmid SCP1. In this paper, the gene has  
been more precisely located by sub-cloning, and the nucleotide  
sequence of the whole fragment has been determined. The predicted  
mmr-specified protein (Mr 49238) would be hydrophobic, with some  
homology at the amino acid level to **tetracycline**  
-resistance proteins from both Gram-positive and Gram-negative  
bacteria. Comparisons of hydropathy plots of the amino acid  
sequences reinforces the idea that the proteins are similar. It is  
suggested that Mm resistance may be conferred by a membrane protein,  
perhaps controlling efflux of the **antibiotic**. No  
significant homology was detected by hybridisation analysis between  
mmr and a cloned oxytetracycline (OTc)-resistance gene (tetB) of the  
OTc producer Streptomyces rimosus, and no cross-resistance was  
conferred by these **genes**. Sequences on both sides of  
**mmr** appear to encode proteins. The direction of translation  
in each case would be opposite to that of mmr translation. This  
suggests that mmr is transcribed as a monocistronic mRNA from a  
bidirectional promoter. An extensive inverted repeat sequence  
between the stop codons of **mmr** and the converging  
**gene** may function as a bidirectional transcription  
terminator.

L13 ANSWER 14 OF 15 MEDLINE DUPLICATE 4  
ACCESSION NUMBER: 87258016 MEDLINE  
DOCUMENT NUMBER: 87258016 PubMed ID: 3037367  
TITLE: Comparison of the rep-38 and mmrA1 mutations of  
Escherichia coli.  
AUTHOR: Sharma R C; Smith K C  
CONTRACT NUMBER: CA02896 (NCI)  
SOURCE: MUTATION RESEARCH, (1987 Jul) 184 (1) 23-8.  
Journal code: 0400763. ISSN: 0027-5107.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English

Searcher : Shears 308-4994

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FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198708  
ENTRY DATE: Entered STN: 19900305  
Last Updated on STN: 19970203  
Entered Medline: 19870805

AB The rep-38 and mmrA1 mutations are located very close to each other (approximately 85 min), and have been suggested to be allelic. To address this question we have compared the phenotypes of the mmrA1 and rep-38 mutants. Both the mmrA1 and rep-38 mutations blocked the enhanced killing and inhibition of postreplication repair by rich growth medium that occurs in UV-irradiated Escherichia coli K-12 uvrA cells, i.e., the mmrA1 and rep-38 strains did not show minimal medium recovery (MMR). However, phi X174 bacteriophage propagated well in mmrA1 strains, but not in rep-38 strains; a rep mutation sensitized a uvrA strain to UV irradiation, but a mmrA mutation did not. During chloramphenicol treatment, the rep-38 strain showed a larger amount of residual DNA synthesis than observed in the mmrA1 strain. The mmrA1 mutation appears to be a dominant mutation. This was determined by the failure of either plasmid pLC44-7 or episome F'KLF11, both of which carry the mmrA+ gene, to complement the Mmr- phenotype of a uvrA mmrA strain. Plasmid pLC44-7 is known to complement the rep-38 mutation, suggesting that rep-38 is a recessive mutation. Although certain of the phenotypes of the rep and mmrA mutants are similar, a number are quite different. These differences suggest that these two mutations are not allelic.

L13 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1984:64861 BIOSIS

DOCUMENT NUMBER: BR26:64861

TITLE: MOLECULAR CHARACTERIZATION OF GENE E  
INVOLVED IN MISMATCH REPAIR IN  
STREPTOCOCCUS-PNEUMONIAE.

AUTHOR(S): CLAVERYS J P; GHERARDI M

CORPORATE SOURCE: CRBGC-CNRS 31062 TOULOUSE CEDEX, FR.

SOURCE: 12TH ANNUAL UCLA (UNIVERSITY OF CALIFORNIA-LOS  
ANGELES) SYMPOSIUM ON CELLULAR RESPONSES TO DNA  
DAMAGE, APR. 10-15, 1983. J CELL BIOCHEM, (1983) 0 (7  
PART B), 220.  
CODEN: JCBSD7.

DOCUMENT TYPE: Conference

FILE SEGMENT: BR; OLD

LANGUAGE: English

(FILE 'HCAPLUS' ENTERED AT 14:39:38 ON 06 JAN 2003)

L8 8 SEA FILE=REGISTRY ABB=ON PLU=ON (QUINILONE OR AMINOGLYC  
OSIDE OR MAGAININ OR DEFENSIN OR TETRACYCLINE OR  
".BETA.-LACTAM" OR MACROLIDE OR LINCOSAMIDE OR SULFONAMID  
E OR SULPHONAMIDE OR CHLORAMPHENICOL OR NITROFURANTOIN  
OR ISONIAZID)/CN  
L14 1116 SEA FILE=HCAPLUS ABB=ON PLU=ON (MMR(10A) (MISMATCH?  
REPAIR?) OR MISMATCH? REPAIR?) (5A) GENE  
L15 4 SEA FILE=HCAPLUS ABB=ON PLU=ON L14 AND (L8 OR QUINILONE  
OR AMINOGLYCOSIDE OR AMINO GLYCOSIDE OR MAGAININ OR  
DEFENSIN OR TETRACYCLIN? OR TETRA CYCLIN? OR BETA LACTAM  
OR MACROLIDE OR LINCOSAMIDE OR SULFONAMIDE OR SULPHONAMID  
E OR CHLORAMPHENICOL OR NITROFURANTOIN OR NITRO FURANTOIN  
? OR ISONIAZID?)

09/912697

L14 1116 SEA FILE=HCAPLUS ABB=ON PLU=ON (MMR(10A) (MISMATCH?  
REPAIR?) OR MISMATCH? REPAIR?) (5A) GENE  
L16 6 SEA FILE=HCAPLUS ABB=ON PLU=ON L14 AND (MULTIANTIBIOT?  
OR ANTIBIOT?)

L17 0 (L15 OR L16) NOT (L1 OR L3 OR L11)

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH,  
JICST-EPLUS, JAPIO' ENTERED AT 14:42:48 ON 06 JAN 2003)

L18 8 S L15 OR L16

L19 0 S L18 NOT (L4 OR L12)

FILE 'HOME' ENTERED AT 14:43:44 ON 06 JAN 2003